New Age Agricultural Bioinputs

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14.1 Introduction

Nitrogen-based biofertilizers are significant bioinputs, but according to current environmental changes and ever-increasing food demand, it is the need of time to popularize more efficient bioinputs for soil. These bioinputs will help to fight against problems like an unpredictable monsoon, global warming, and decreasing soil fertility, and indiscriminate use of agrochemicals.

Besides chemical fertilizers, organic soil conditioners, the application of phosphate solubilizers, nitrogen fixers, and *Trichoderma*, *Verticillium*, *Metarhizium* like versatile biocontrolling agents are the common strategies of soil conditioning. In the past 50 years, there is tremendous work published on nitrogen fixers and phosphate solubilizers. The results of these findings directed to the exploitation of common biofertilizers like *Azotobacter* and *Rhizobium* as a nitrogen fixer and other organic inputs. In addition to above, phosphate, zinc, sulphur, potassium solubilizers are a

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significant part of current agricultural practices. Although these practices proved beneficial to uphold soil fertility and other agronomical problems like pest attack and plant susceptibility to various infections, physiological problems due to the change in the atmosphere need some novel strategies or additional bioinputs.

There are various significant bioinputs like the application of 1-aminocyclopropane-1-carboxylic acid (ACC) enzyme and phytase producing microorganisms and bacterivorous flora. These are which were reported, but unfortunately remain as neglected practices by Indian farmers. The following three major bioinputs are need of time to use as new soil bioinputs in modern agricultural practices:

1. Use of ACC oxidase and deaminase producer bioinputs
2. Use of phytase producer
3. Use of bacterivorous soil microbes

The central idea of this chapter is presented in Fig. 14.1, which represents the ability of major modern agricultural bioinputs.

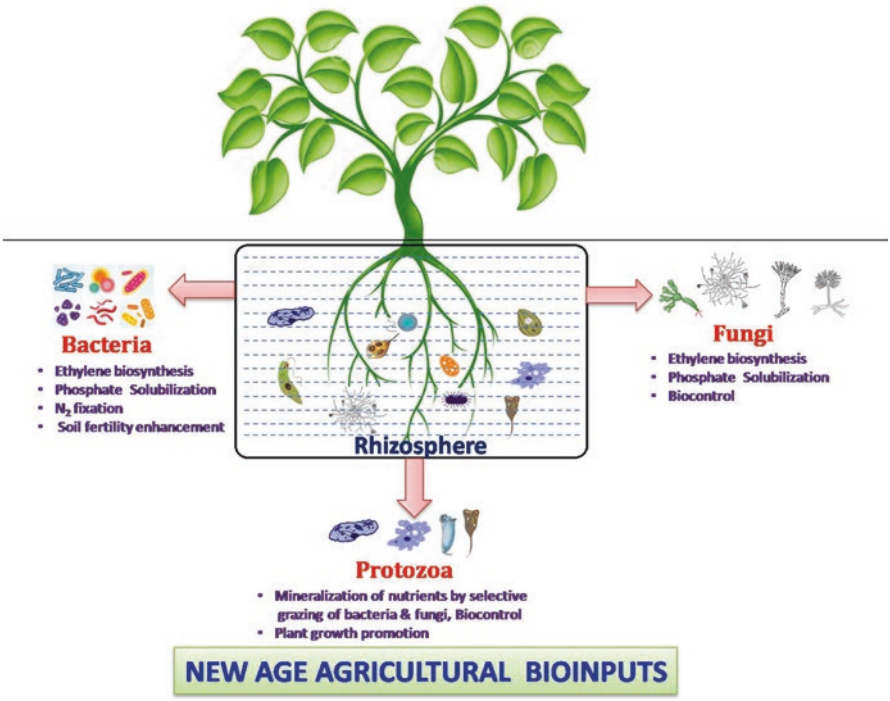

Fig. 14.1 Schematic representation for the new age agricultural bioinputs

14.2 Application of ACC Oxidase and Deaminase Producer Bioinputs

14.2.1 ACC and ACC-Degrading Enzymes

The Yang cycle produces 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC oxidase and deaminase (ACCO and ACCD) (Yang and Hoffman 1984). Shang Yang unlocked the mystery of freshness of fruit, flowers, defoliation, and ripening of fruits by proposing a continuous biochemical cycle known as the Yang cycle. The Yang cycle biosynthesizes ethylene in plants. Ethylene is important in host–pathogen interactions, seed germination, flowering, and fruit ripening. It establishes the central role of methionine in ethylene synthesis. Yang's study proved the genesis of S-adenosylmethionine as a transitional compound which is further converted into ACC and then ethylene (Fig. 14.2).

ACC is the signaling molecule of a plant, easily transported through intra- and intracellular tissues over short and long distances.

ACC is a cyclic α-amino acid with a three-membered cyclopropane ring merged to an α-carbon atom of the amino acid (Fig. 14.3) and chemical formula $C_4H_7NO_2$ with a molar mass of 101.0 g/mol^{-1}. ACC is considered an essential intermediate that regulates ethylene biosynthesis. The enzyme ACCO is a member of the oxidoreductase class, which is responsible for the transformation of

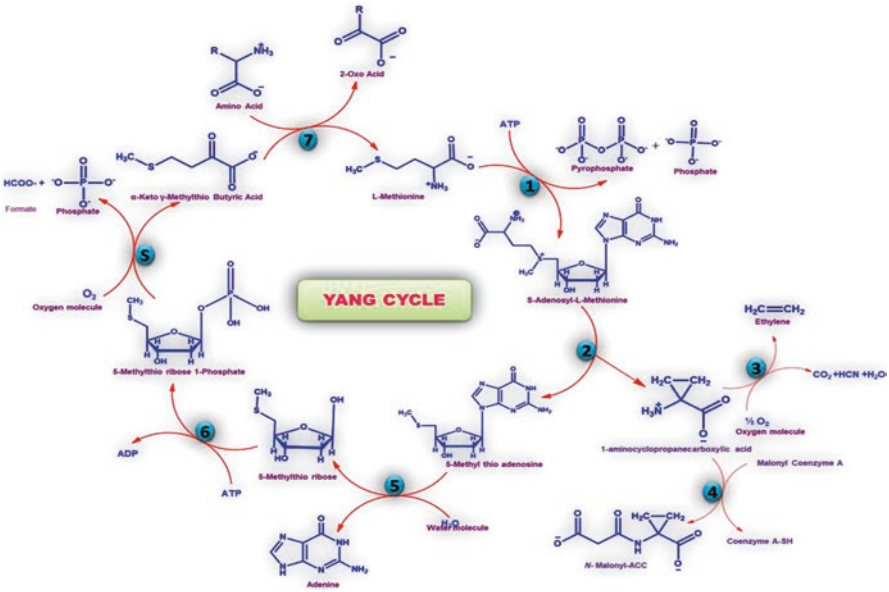

Fig. 14.2 Yang cycle for ethylene biosynthesis. Cycle path: (1) SAM synthetase, (2) ACC synthase, (3) ACC oxidase, (4) ACC N-malonyltransferase, (5) MTA nucleosidase, (6) MTR kinase, and (7) transaminase, (S) spontaneous reaction

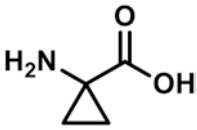

Fig. 14.3 Chemical structure of ACC

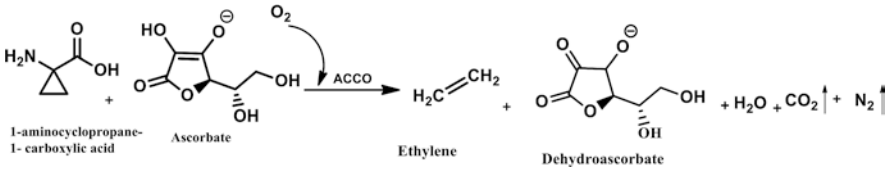

Fig. 14.4a Transformation of ACC to ethylene with ACCO

1-aminocyclopropane-1-carboxylate to ethylene with carbon dioxide, water, and other by-products (Fig. 14.4a).

In drought stress conditions, ethylene synthesis is rapidly increased (Morgan and Drew 1997). Ethylene is the one of the marker compounds of drought conditions and is also known as stress ethylene. Nitrogen fixation and nodulations are influenced by the various effects of high ethylene synthesis through water and temperature stress, like reduction of transpiration rate by closing stomata to regulate the abscisic acid pathway (Tanaka et al. 2005; Tamimi and Timko 2003; Penmetsa and Cook 1997; Guinel 2015). Hence, if the ACCO is regulated, then the natural synthesis of ethylene is regulated. Various researchers advocated that various rhizospheric microbes also control the ethylene level in a plant by deaminating ACC diffused through root cells and seeds (Finlayson et al. 1991; Penrose and Glick 2001; Penrose and Glick 2003).

14.2.2 Aminocyclopropane-1-Carboxylic Acid Oxidase (ACCO)

Aminocyclopropane-1-carboxylic acid oxidase is an enzyme recognized to fight against the consequences of drought in plants. It was well documented that drought affects various biochemical, morphological, and physiological activities of plants, e.g., turgor pressure, transport of soil nutrients, nutrient transport to root, nutrient diffusion through root mass, and a run of water-soluble nutrients such as silicon, manganese, and sulphate. Besides these, it leads to oxidative stress, which causes a decrease in chlorophyll synthesis, membrane deterioration, and protein degradation in plants (Hsiao 2000; Selvakumar et al. 2012; Sgherri et al. 2000; Rahdari et al. 2012).

14.2.3 Aminocyclopropane-1-Carboxylic Acid Deaminase (ACCD)

ACCD is the enzyme synthesized in the cytoplasm of bacteria. It is a multimeric sulfhydryl enzyme having a monomeric subunit with molecular weight of 35–42 KD (Glick et al. 2007). ACCD catalyses ACC conversion and produces α-ketoglutaric acid and ammonia (Fig. 14.4b). It was reported that D-serine and D-cysteine (D-amino acids) also act as a substrate for ACCD. Previously, the optimum temperature and pH for ACC deaminase were reported as 30–35 °C and 8.5 (Jacobson et al. 1994; Honma and Shimomura 1978; Jia et al. 1999). But currently, there is significant research going on to screen a versatile ACC deaminase producer who has a broad temperature and pH range (Xuguang et al. 2018). Various bacteria were reported for the production of ACCD, e.g., *Enterobacter cloacae*, *Pseudomonas putida*, *Pseudomonas* sp., *Alcaligenes*, *Hansenula*, *Rhizobium*, *Sinorhizobium* sp., *Pseudomonas chlororaphis*, *Rhizobium leguminosarum*, and *Bacillus subtilis* (Klee et al. 1991; Glick 1995; Belimov et al. 2007; Tittabutr et al. 2013; Ma et al. 2004; Duan et al. 2009). Similarly, some fungi and yeast were also reported for ACCD production, e.g., *Penicillium citrinum* (Minami et al. 1998; Jia et al. 1999).

Glick (1995) described the role and importance of some plant growth–enhancing *Rhizobacterium* in the management of drought pressure and various physiological activities of plants. Glick (1995) illustrated that ACC is produced in more quantity during drought stress and exudated outside of the root cells. The plant growth-inducing bacteria around the roots are recognized for its versatile activity and utilize the ACC exudate by ACC deaminase, and to keep the balance in internal and external ACC level, internal ACC is transported outside of the root. This process reduces the amount of ACC required for the biosynthesis of ethylene inside plant cells. Hence, if such ACCD-producing *Rhizobacterium* is present around the rhizospheric area of vegetation in a drought condition, ethylene production is suppressed, further leading to restrain inhibitory stress; ethylene causes defoliation, inhibition of root elongations, and nodulation transpiration (Glick et al. 2007). The presence of ACCD-producing microbes in soil proved their significance in a variety of plant growth–promoting activities, e.g., the existence of ACCD producer enhances the nitrogen fixations by inducing the normal process of root nodule organization in drought or temperature stress conditions.

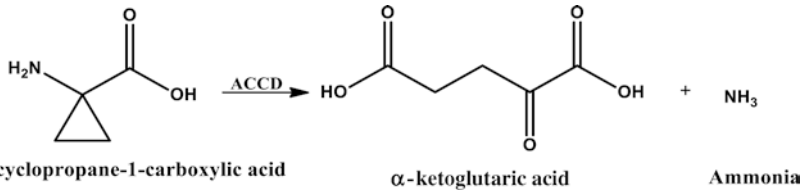

Fig. 14.4b Conversion of ACC to ethylene with ACCD

14.3 Application of Phytase Producer

14.3.1 Importance of Phosphorous

Phosphorous (P) is the next main macronutrient required for plant growth after nitrogen. It accounts for about 0.2% of dry weight of a plant. It makes vital biomolecules like nucleic acids, ATP, and phospholipids, and ultimately plant growth is inhibited without the supply of this nutrient. It also has a role in the regulation of the metabolic pathway and enzyme-catalyzed reactions. Phosphate affects germination and seed maturity and eventually plant development. Plant development comprising of root, stem, and stalk is dependent on phosphate. Phosphate has a role in the formation of seed and flower, which ultimately has an effect on crop development and yield (Khan et al. 2009). It has a remarkable function in N fixation in legumes, energy metabolism, membrane synthesis, photosynthesis, respiration, enzyme regulation, crop value, and abiotic and biotic stress resistance. No atmospheric source of phosphate could be made available to plants (Ezawa et al. 2002), and soils normally contain trace quantities of available phosphate (predominantly as HPO_4^{2-} and $H_2PO_4^-$) that is readily available for plant uptake. Phosphate addition in the soil in the form of fertilizers fulfills the plant requirement (Richardson et al. 2009). The unavailability of phosphate in soluble form is a vital factor (Xiao et al. 2011) that restricts the agricultural production worldwide (Ramaekers et al. 2010). Both organic and inorganic phosphate accumulate in soil and consequently not available for plant consumption. Inorganic phosphate is fused through chemical adsorption and precipitation, while immobilization of organic phosphate occurs in soil organic matter (Sharma et al. 2012).

Even phosphatic fertilizers fail due to their conversion to an insoluble form like calcium phosphate and aluminum phosphate (>70%) (Mittal et al. 2008). Phosphate is available in low quantity in soil (1.0 mg kg^{-1} soil); additionally, it becomes unavailable by reacting with reactive metals like Al^{3+} in acidic, calcareous, or normal soils (Gyaneshwar et al. 2002; Hao et al. 2002). Crop plants can, therefore, make use of only a little bit of phosphorus, which eventually results in reduced crop performance (Reddy et al. 2002). The high percentage of an insoluble type of phosphate leads to eutrophication, while frequent use of phosphate causes soil infertility and rapid depletion of nonrenewable phosphate reserves. The outcome of this event would be the lake's biological death i.e. cyanobacterial blooms, hypoxia, and death of aquatic animals due to depleted bioavailable oxygen and buildup of nitrous oxide. (Vats et al. 2005). In the plant, a range of morphological and physiological changes was observed due to deficiency in phosphate, which consecutively affects plant growth, productivity, and survival (Tran et al. 2010), and hence are a significant pin down for the agriculture industry worldwide.

Hence, effective phosphorous utilization is crucial for the sustainable expansion and prevention of undesirable environmental effects (Scholz et al. 2015). The translation of a phytate–phosphate compound in the soil in crop accessible orthophosphate would mitigate phosphate-related obstacles.

14.3.2 What Is Phytate?

Phytate is a significant storage compound of phosphorus in seeds. Eighty percent of the total seed phosphorus is made by phytate, which accounts for 1.5% of seed dry weight (Raboy and Dickinson 1987). The myo-inositol hexakisphosphate is a phosphate salt of myo-inositol having all six hydroxyl groups substituted by phosphate residues (Fig. 14.5). The myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen) phosphate is commonly called myo-inositol hexakisphosphate, or phytate, which is a collection of the organic form of phosphorus compounds found widely in nature. The prefix "hexakis" designates that the phosphates are not internally connected and the compound is formed by a polydentate ligand, which binds with more than one metal atom coordination site. Each phosphate group is in ester form within an inositol ring and binds entirely with 12 protons (Bohn et al. 2008; Cao et al. 2007).

Phytate usually presents as a salt of monovalent and divalent cations (Fe^{2+}, Mn^{2+}, K^{+}, Mg^{2+}, and Ca^{2+}) and formed in seeds at the stage of ripening. In phytic acid, the negatively charged phosphate sturdily binds with positively charged metallic cations resulting in an insoluble complex and restricting the accessibility of nutrients. Phytic acid and its derivatives are accountable for various cellular events such as signaling, RNA export, endocytosis, DNA repair, and vesicular cell trafficking (Bohn et al. 2008; Frias et al. 2003). In plants, phytate is the prime storage type of inositol phosphate. The plant root has 30% phosphorus fractions, while seeds and cereal grains have 80% phosphorus (Lott et al. 2000; Turner et al. 2002; Haefner et al. 2005). Two pathways are considered for the biosynthesis of phytate: lipid-dependent and lipid-independent. The synthesis of phytic acid starts from myo-inositol via a series of phosphorylation steps. In the former route, phytate is attained by the successive phosphorylation of Ins(1,4,5)P3 (inositol 1,4,5-triphosphate) and Ins(1,3,4)P3 (inositol 1,3,4-triphosphate). The subsequent compound is released from PtdIns(4,5)P2 (phosphatidylinositol 4,5-biphosphate) by the effect of a specific phospholipase C. The intracellular location of the intermediates of phytic acid biosynthesis is not fully explored.

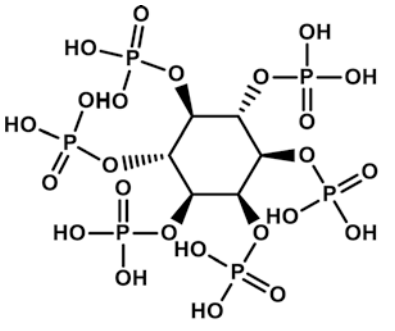

Fig. 14.5 Structure of phytate

Organic phosphate in rhizosphere has a high affinity to soil particles by precipitation and adsorption and hence it creates deprived accessibility to the plant as it cannot be desorbed (Menezes-Blackburn et al. 2013). Phytic acid is degraded in seed germination by a precise assembly of enzymes called phytases.

14.3.3 Phytase Enzyme

Phosphorus deficiency results from the phytase secretion of a variety of plant roots (Minggang et al. 1997). The distinct phosphatases phytases (myo-inositol hexakisphosphate phosphohydrolase) sequentially hydrolyze the phosphomonoester bonds from phytic acid, thereby liberating lower inositol phosphates and inorganic phosphate (Singh et al. 2011). These catalysts commence phytic dephosphorylation at various positions on the inositol ring, and it produces diverse isomers of lower inositol phosphates (Turk et al. 2000).

14.3.4 Structure and Mechanism of Action of Phytase

Phytase (myo-inositol hexakisphosphate phosphohydrolase) is a homodimaeric enzyme (EC 3.1.3.26 and EC 3.1.3.8) (Hegeman and Grabau 2001; Guimarães et al. 2004). Phytases carried out the subsequent release of inorganic phosphorus from phytic acid. Phytases act hydrolytically to break the phosphate ester bond of phytate and release inositol phosphates and phosphorus with other essential nutrients, which are required for plant absorption (Angel et al. 2002) (Fig. 14.6). Phytases are involved in the dephosphorylation of inositol-6-phosphate and high-order inositol hexakisphosphate hydrolyze sequentially to form lower-order esters like inositol monoesters (Hayes et al. 1999; Vats and Banerjee 2004). The inositol penta- and hexakisphosphate (phytate) hydrolyzing enzymes are of interest because they constitute a high percentage of the whole organic phosphate (Turner et al. 2002).

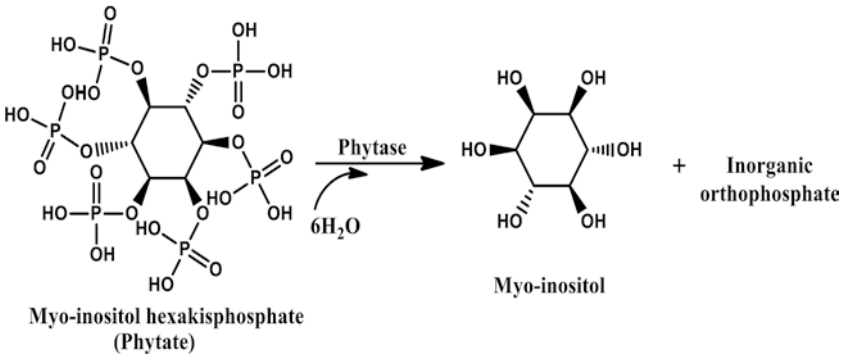

Fig. 14.6 Phytase action on phytate

The phytase protein has substrate binding and catalyzation conserved domains. The substrate binding domain is present at the N-terminal with RHGxRxP conserved sequence for substrate binding. The C-terminal catalyzation theme comprises of particular HD components. The "pocket" structure is framed by the connection of residues in the motif (Mullaney et al. 2000). The substrate restricting site with RHGxRxP arrangement responds with the substrate and frames the chemical substrate complex. The phosphate groups are then released from the substrate by the HD element (Li et al. 2010).

Phytate hydrolysis occurs in two stages: the nucleophilic attack and protonation. The histidine in the dynamic site of the catalyst caused a nucleophilic assault to the fragile phosphoester bond of phytate and caused the protonation by the aspartic acid of the leaving cluster (Li et al. 2010). The ß-propeller alkaline phytases lack the RHGXRXP sequence motif, and hence it needs calcium thermostability as well as enzyme activity to produce the IP3 (inositol triphosphate) (Kim et al. 1998a; Mullaney and Ullah 2003).

Phosphatases cause hydrolysis of 60% of the total organic phosphate. The highest quantity of phosphate was released by phytases from phytate (Bünemann 2008). The release of orthophosphate from soil natural phosphate is effective in microbes as well as in plants. Plant phytases have been distinguished in roots and root exudates during the early stage of seed germination; they frequently show a poor action, making them inefficient for hydrolyzing soil phytic acid as well as phosphorous usage (Hayes et al. 1999; Richardson et al. 2009) and thus suggest that the microbial catalyst demonstrates superior, effective liberation of phosphorous (Tarafdar et al. 2001).

14.3.5 Categorization of Phytases

Phytases are assembled by their enzyme action, pH action, and the initiation site of dephosphorylation of phytate. They are categorized into 3-phytases (EC 3.1.3.8), 5-phytases (EC 3.1.3.72), and 6-phytases (EC 3.1.3.26) on account of the initial hydrolysis position of phytate according to IUPAC-IUBMB (Bohn et al. 2008), which were subsequently alienated into alkaline and acid phytases (Jorquera et al. 2008). The three-dimensional structure and catalytic mechanism cause classification into four classes: histidine acid phytases (HAP) (EC 3.1.3.2), cysteine phytase or purple acid phosphatase (PAP) (EC 3.1.3.2), beta-propeller phytase (BPP) (EC 3.1.3.8), and protein tyrosine phosphatase (PTP)-like phytases (Li et al. 2010), which have recently been characterized (Lei et al. 2007). HAPs and BPPs are the most well-known and contemplated phytases. Various bacterial, fungal, and plant phytases have a place with the HAP family, while BPP has all the earmarks of being the prevalent phytase in *Bacillus* species (Greiner et al. 2007; Huang et al. 2009). These two most important categories have a different catalytic activity that results in distinct end products. While HAPs catalyze the hydrolysis of PA in myo-inositol and Pi, BPP activity results in the creation of the inositol-triphosphates – either Ins(1,3,5)P3 or Ins(2,4, 6)P3 (Greiner et al. 2007; Kerovuo et al. 2000).

As per the optimum pH, acid phytases, for the most part, incorporate HAP, PAP, and PTP-like phytases, though alkaline phytases include just BPPs from *Bacillus* species (Singh and Satyanarayana 2015; Tye et al. 2002). Alternatively, carbon position of dephosphorylation initiation resulted in phytases grouping into 3-phytase (myo-inositol hexakisphosphate 3-phosphohydrolase), 6-phytase (myo-inositol hexakisphosphate 6-phosphohydrolase), and 5-phytase (myo-inositol hexakisphosphate 5-phosphohydrolase).

The categorization of phytase into EC 3.1.3.8, EC 3.1.3.26, and EC 3.1.3.72 (myo-inositol-hexaphosphate phosphohydrolases) was organized on the background of protein sequencing, and successive dephosphorylation (George et al. 2007) of P occurs at three and six positions, correspondingly. The labeling basis is the three- and six-bond position of myo-inositol 6-phosphate. The 3-phytases (EC 3.1.3.8) are present in filamentous fungi like *Aspergillus* sp. and 6-phytases (EC 3.1.3.26) are found in plants, e.g., wheat.

14.3.6 Reserve of Phytase

Phytases can be formed by microorganisms, plants, and animals. Wheat, rice, soybeans, barley, peas, corn, and spinach are examples of plant sources. Microorganisms like bacteria, fungi, and yeast are the real source of phytase found in the blood of vertebrates such as fish and reptiles (Gupta et al. 2015; Bohn et al. 2008). Among the phytases from microorganisms, attention is focused on *Aspergillus* sp. because of its high production and extracellular activity (Gupta et al. 2015). To circumvent this obstacle the sole strategy is the application of phytases which hydrolyze the phytate and increase availability of P to plants. Commercially available phytase addition is costly and time-consuming, and hence the maintenance of rhizospheric phytase producer is important. Another engineering approach involves incorporation of genes behind phytase production from microbes into transgenic plants. However, there is a range of constraints for phytase engineered crop plants like loss of seed viability, yield, vulnerability for ecological pressure, and rejection of genetically modified organisms (GMOs) (Reddy et al. 2017).

14.3.7 Microorganisms Producing Phytase

Phytases of microbial origins are of rigorous significance among plants, animals, and microorganisms owing to the ease of genetic manipulation and large-scale production (Adhya et al. 2015). Microorganisms are the key drivers in the soil, which regulates phytate mineralization. The occurrence of microorganisms in soil rhizosphere may balance plants inability to procure P directly from phytate. In microorganisms, bacteria, yeast, and fungi have been effectively researched for extracellular phytase action (Pandey et al. 2001). A single phytase cannot address the issues of business and ecological applications (Bakthavatchalu et al. 2013). Microbial

phytases are investigated mainly from fungi of a filamentous type such as *Aspergillus ficuum* (Gibson 1987), *Mucor piriformis* (Howson and Davis 1983), *Aspergillus fumigatus* (Pasamontes et al. 1997), *Cladosporium* sp. (Quan et al. 2004), and *Rhizopus oligosporus* (Casey and Walsh 2004). Phytase production by different bacteria has been described, viz., *Bacillus* sp. (Kim et al. 1998b; Choi et al. 2001), *Citrobacter braakii* (Kim et al. 2003), *Pseudomonas* sp. (Richardson & Hadobas 1997), *Escherichia coli* (Greiner et al. 1993), *Raoultella* sp. (Sajidan et al. 2004), and *Enterobacter* (Yoon et al. 1996). The anaerobic rumen bacteria, mainly *Selenomonas ruminantium*, *Prevotella* sp., *Megasphaera elsdenii*, and *Mitsuokella multiacidus* (Richardson et al. 2001b) and *Mitsuokella jalaludinii* (Lan et al. 2002), have also been investigated for phytases. The γ-proteobacteria group possesses the phytase production potential among the majority of soil bacteria. Fungi have extracellular phytases, while bacteria produce cell-linked phytases. *Bacillus* (Choi et al. 2001; Kerovuo et al. 1998; Kim et al. 1998a; Powar and Jagannathan 1982; Shimizu 1992) and *Enterobacter* (Yoon et al. 1996) are the only bacterial genera having extracellular phytase activity. The phytase activity of *Selenomonas ruminantium* and *Mitsuokella multiacidus* (D'Silva et al. 2000) is outer membrane linked, while *Escherichia coli* produces the periplasmic phytase enzyme (Greiner et al. 1993).

B. subtilis is as a competent of phytase producer owing to its nonpathogenic and safe nature for industrial-level phytase production. This microorganism has numerous additional advantageous properties like organic acid production and antibiosis for phosphate solubilization in the soil. Currently, *Aspergillus* and *E. coli* are the commercial phytase producers. Among the various organisms reported, the inhabitant *E. coli* enzyme demonstrates the maximum phytase activity.

Phytases from bacterial sources are a genuine option in contrast to fungal enzymes because of their specificity to the substrate, protection from proteolysis, and effective catalytic action (Konietzny and Greiner 2004). *Bacillus* phytases are exceptionally effective due to its higher thermal stability and neutral pH. The *Bacillus* phytase has stringent specificity for a substrate for the calcium–phytate complex effective for application in the environment (Farhat et al. 2008; Fu et al. 2008). Nevertheless, owing to inefficient enzyme production methods for *Bacillus* sp., it could not be produced at commercial scale as only a few strains have been significantly commercialized for phytase production (Zamudio et al. 2001). *Lactobacillus sanfranciscensis* is the main sourdough lactic acid bacteria that demonstrated a significant level of phytate degrading action (De Angelis et al. 2003). The HAP are specifically produced from *Aspergillus* sp. like *A. terreus*, *A. ficuum*, and *A. niger* (Wyss et al. 1999), while the alkaline phytases are produced from *Bacillus amyloliquefaciens* (Idriss et al. 2002) and *Bacillus subtilis* (Kerovuo et al. 2000). Escobin-Mopera et al. (2012) had purified phytase from *Klebsiella pneumoniae* 9–3B. Rhizobacteria can mineralize phytate and may enhance P uptake of plants in soils (Patel et al. 2010). A better and substitute resource of phytase is continuously searched by screening new organisms that may produce novel and effective phytases. The ultimate aim is to produce phytase cost-effectively with optimized conditions for industrial application.

14.3.8 Why Do Bacteria Produce Phytase?

Bacterial phytase production is an inducible complex regulatory mechanism. Phytase synthesis control is different in various bacteria. Phytase production is not a condition for balanced bacterial growth, but it is the response to an energy or nutrient constraint. Phytase formation takes place when bacterial cells face environmental variations prior to the commencement of growth or when actively growing culture faces a stressful condition. The metabolic regulation by signal transduction is also a mechanistic role (Zamudio et al. 2002).

14.3.9 Parameters Affecting the Activity of Phytases

The soil environment presents extreme difficulties like denaturation, degradation, adsorption, and dilution to extracellular chemicals (Wallenstein and Burns 2011). The constancy of extracellular and intracellular enzymes is variable. Stability is portrayed more in extracellular than intracellular proteins and is credited by glycosylating disulfide bonds that alter thermal soundness, an expansive pH scope of action, and some protection from proteases. Some are stabilized by binding with humic substances and clay minerals (Quiquampoix and Burns 2007). Biological and physicochemical procedures influence phytase action. The former causes changes in enzyme creation rates leading to isoenzyme generation and changes in microbial network synthesis, while the latter causes changes in absorption desorption responses, substrate dissemination rates, and enzyme degradation rates (Wallenstein et al. 2009). Essential elements influence the action of enzyme include the amount and kind of substrate (Fitriatin et al. 2008), type of solvent, pH, temperature, the existence of an inhibitor and activator, the quantity of the enzyme, and the reaction product (Sarapatka 2002).

14.3.9.1 Effect of Substrate on Phytase Action

Phytase action shifts with various substrates. The different substrates include 1-naphthyl phosphate, 2-glycerolphosphate, glucose-6-phosphate (Escobin-Mopera et al. 2012), 2-glycerolphosphate, fructose-6-phosphate, calcium phytate, sodium phytate, p-nitrophenyl phosphate, ß-glycerol phosphate, adenosine-5′-monophosphate (AMP), guanosine-5′-triphosphate (GTP), adenosine-5′-diphosphate (ADP), adenosine-5′-triphosphate (ATP), and nicotinamide adenine dinucleotide phosphate (NADP) (Farouk et al. 2012; Bakthavatchalu et al. 2013). Phytases are categorized as substrate particular and nonparticular acid phosphatases (Rossolini et al. 1998; Rodríguez and Fraga 1999).

14.3.9.2 Effect of pH on Phytase Action

The activity of phytases relies on the pH and temperature. Plant phytases have less pH and thermal stability than microbial phytases. The optimum pH for phytase activity is 5.0–8.0, hence classified as acid or alkaline phytases, respectively (Konietzny and Greiner 2002). The optimum pH for fungal phytases is 4.5–6.5 with

80% activity; for example, *Rhizoctonia* sp. and *F. verticillioides* have an optimum pH of 4.0 and 5.0, respectively (Marlida et al. 2010). The optimum pH for bacterial phytases is 6.0–8.0 (Kerovuo et al. 1998; Kim et al. 1998a). Acidic phytases have an optimum pH range from 4.5 to 6.0 (Konietzny and Greiner 2002), and pH 8.0 is the optimum for alkaline phytases in legume seeds (Scott 1991), lily pollen (Baldi et al. 1988), and cattail (Kara et al. 1985; Scott 1991).

14.3.9.3 Effect of Temperature on Phytase Action

Temperature is the most indispensable factor of enzyme action, influencing both enzyme generation and degradation rates by microorganism. The ideal temperature of phytate-degrading enzyme fluctuates from 35 to 77 °C. Predominantly plant phytases have the greatest action at lower temperature compared to microbial phytases (Konietzny and Greiner 2002). The ideal temperature for plant phytases ranges from 45 to 60 °C (Johnson et al. 2010). In general, metabolic rate of enzyme producing life forms increases with temperature over the range 5–40 °C. In this way, temperature supposes a more vital job in the rate of extracellular enzyme activity when contrasted with enzyme kinetics itself.

14.3.9.4 Effect of Soil Type on Phytase Action

The action of phytase in soil is additionally influenced by physicochemical properties of the soil, which incorporates soil compose, organic matter content, nitrogen content, C/N proportion, and aggregate P content (Djordjevic et al. 2003). The soil performance of phytase fluctuates with soil compose, and the movement of phytase lost expeditiously is dependent on three differentiating soil nature. The initial fate of phytase is confined by adsorption in the soil. The degradation and magnitude of phytase adsorbed continue as before for a wide range of soil arrangements. The highest adsorption was recorded at low pH, and it becomes nearly equivalent to zero when pH is adjusted to 7.5. The adsorption bestows defense to phytase degradation in the soil, but also limits loss of enzyme activity in the adsorbed state.

14.3.10 Mechanism of Phytase Activity

Microorganisms can enhance the capacity of a plant to acquire P through various mechanisms, and the important one is phytase like enzyme production (Richardson and Simpson 2011). The purified crystalline form of phytase has different catalytic properties with specific diverse mechanisms. The principal action of all portrayed phytases depends on the enzymatic hydrolysis of the bonds among inositol and phosphoric acid deposits. Enzymatic hydrolysis of bonds happens among inositol and phosphoric acid deposits whereupon the component of activity of all phytases is based. The results of this arrangement of responses are six-fold alcohol and phosphates (Mukhametzyanova et al. 2012). Microbial phytases decay fresh plant buildups in the soil prompting the release of phosphorus from organic compounds. There are various arrangements alongside differing rates of responses by which the phosphoric acid deposits are discharged through microbial hydrolysis of phytate

(Mukhametzyanova et al. 2012). The histidine acidic phytases catalyze the release of phosphates in neighboring free hydroxyl group, after the dephosphorylation of a first phosphate group. For the most part, plant phytases display a difference in transitional myo-inositol pentaphosphate development among the first phase of the response. In the course of the first venture of hydrolysis, microbial 6-phytases frame a different set of intermediates. The acid phosphatases with phytate hydrolyzing properties hydrolyze glucose-1-phosphate in *Enterobacteriaceae* (Greiner and Sajidan 2008). Alkaline phosphatases in lily pollen, *B. subtilis*, and reed mace formed myo-inositol triphosphates as end products (Greiner et al. 2007; Greiner and Sajidan 2008; Mukhametzyanova et al. 2012).

14.3.11 Importance of Microbes for Phosphorous Mobility with Phytase

Soil microorganisms, particularly the higher plant rhizosphere, are exceptionally powerful in discharging P from natural pools of aggregate soil P by mineralization and inorganic complexes through solubilization (Hayat et al. 2010).

Mineralization results from the transformation of organic P, for example, phytate to plant-accessible inorganic P, by microorganisms through their expressed enzyme phytase (Ariza et al. 2013). Phytases have been recognized in roots and root exudates in plants (Li et al. 1997; Hayes et al. 2000; Richardson et al. 2000). Despite the fact that it is accounted for the enzymatic action in root exudates, it is not sufficient for efficient use of natural phosphorous (Brinch-Pedersen et al. 2002; Richardson et al. 2000). The addition of exogenous phytase into the media resulted in phytate availability for plant growth (Hayes et al. 2000; Idriss et al. 2002; Unno et al. 2005). The addition of exogenous phytase (Idriss et al. 2002; Richardson et al. 2001b; Singh and Satyanarayana 2010; Hayes et al.2000) or expression of phytase gene of microbial origin in plant (Richardson et al. 2001a; Li et al. 2007a, b, 2009) resulted in growth of plant with phytate as solitary source of phosphate. The current research is targeted on the genetic expression of phytase genes in the plant for organic P utilization from the soil. The graphic demonstration of the function of microorganisms in phosphate solubilization is described in Fig. 14.7.

The action of plant phytases comprises just a little extent of the aggregate phosphatase reaction and is viewed as insufficient for guaranteeing adequate phosphate securing (Richardson et al. 2000; Findenegg and Nelemans 1993; Hayes et al. 2000). Bacterial phytases are effective for growth and yield of the plant. The limitation of plants to extort P from soil phytate could be overcome by treatment with phytate-degrading bacteria, like biofertilizer. Microbial phytase plays a very important role for the availability and mobility of phosphorous in soil because of its agronomic and ecological value for the growth of the plant as suggested by the recent scientific research. The long-term phosphorous deprivation in plants could be met by phytase from microorganisms; hence, the use of microbial phytase on an industrial scale is very appealing nowadays (Jorquera et al. 2008). The fungal extracellular phytase-treated seeds support the plant phosphorus nutrition in high phytate

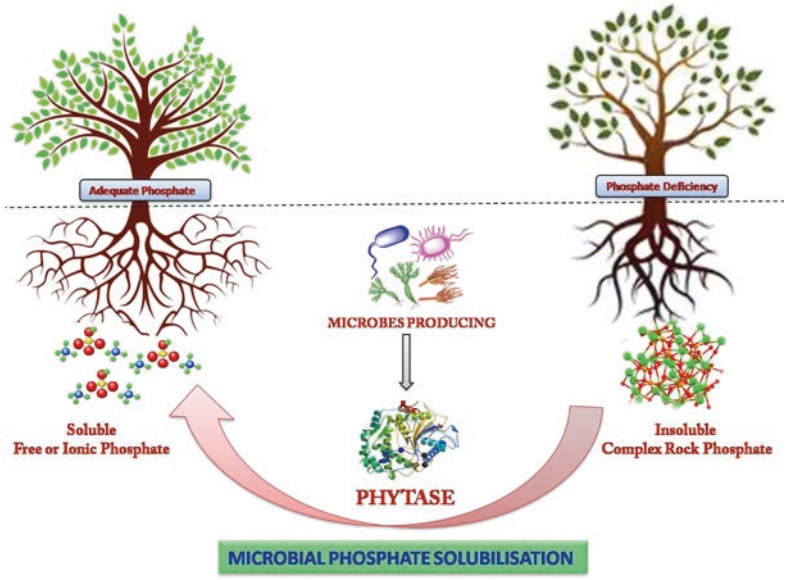

Fig. 14.7 Role of phytase from microorganisms in phosphate solubilization

content soil (Tarafdar 1995). The enrichment of soil with phytase from bacteria like *B. amyloliquefaciens* and *Bacillus mucilaginosus* advances the development of corn and tobacco, respectively (Li et al. 2007a, b; Idriss et al. 2002). Phytases from bacteria also release the vital soil micronutrients by phytate chelation and make it available to the plant. The purified microbial phytase or phytase-producing microbial strains could be functional as an effective and eco-friendly way to increase bioavailable soil phosphorus and limit the wide utilization of inorganic phosphate fertilizers.

14.3.11.1 Transgenic Plants for Phytase

Gene for phytase from a microorganism is integrated into plants like tobacco with a phyA gene from *A. niger* constituting phytase as soluble proteins in tobacco seeds. Genetically modified plants produce extracellular phytase from roots, which showed significant improvement in P nutrition in the soil, with higher phytate content or artificially modified for phytate (George et al. 2004, 2005). Thus the phytase from a microorganism is the critical element, and their existence in the rhizosphere helps the plant to recover from its inability to use the unavailable phytate.

Phytases have developed to be a valuable key to supportable agribusiness. It gives an approach to stop the revenue costs that turn out to be superfluously high because of the expansion of phosphorus manures. Broad research on phytase utilizing biotechnological applications will unquestionably give efficient arrangements towards practical agribusiness and ecological insurance in the coming years.

14.4 Use of Bacterivorous Microbes from Soil

14.4.1 Bacterivorous Protozoan

It was an accepted truth that soil microbes provide essential functions supporting soil fruitfulness and plant well-being. Recent evolution in molecular techniques like molecular sequencing resulted in a boom in studies of various microflora like an insect, animal gut, lakes, ponds, and terrestrial flora. However, all these studies cover bacteria and fungi only and neglect other trophic levels. But most attempts to use these bacteria and fungi as bioinputs in natural soil have been reported unsuccessful.

For the past 50 years the terms "biofertilizer" and "PGPR bacteria" only represent nitrogen fixer and phosphate and growth hormone producer. However, the truth is there is still no confirmation that these added bioinputs sustain soil fertility. The accepted truth is that these fungal and bacterial bioinputs have significant selective pressures of predation and not resource availability. These predators are bacterivorous and fungivorous protist. Protists massively consume bacteria as well as other soil microbes like fungi and yeast, and unicellular algae and release various micronutrients, growth-promoting substances, and different assimilable nitrogenous compounds and mineral (Ekelund and Rønn 1994).

Although various soil protozoans and nematodes are reported for their bacterivorous role, very few reports exist discussing the function of protozoans in the development of crop plant or soil richness (Bonkowski and Brandt 2002; Bonkowski 2004). The size of most soil protozoan ranges from 10 to 100 μm in diameter, but their weight is negligible. It was assumed that the biomass of total protozoan in soil is equal to the biomass of all other clusters of soil animals together except earthworm (Schaefer and Schauermann 1990; Schröteret al. 2003). In the biological energy coordination, the soil organic cycle plays an important role, which involves anabolic and catabolic steps of energy investment and energy escape or lost. Protozoans are major engineers which motion this organic energy cycle in the soil. Protozoa drive this cycle continuously where there is sufficient water available like moisture-containing intersoil capillaries, pore spaces, and fissures. Besides these, protozoans account for significant respiration of soil. It was noted that they contribute to 15–70% of the entire soil respiration. These indicate that protozoans are a vital component of the soil. The soil protozoans majorly include ciliates, flagellates, and naked and testate amoebae (Fig. 14.3). Although these protozoans have an extensive array of food assimilation and enzyme syntheses like a higher animal, they are not capable of synthesizing some vitamins and cofactors, and hence they depend on some microbial population for it.

Ciliates are one of the group including protozoan, which are identified for its extraordinary bacterivorous capacity (Sherr et al. 1987); owing to their large size. Algae, fungi, and small animals are foods for these ciliates (Bernard and Rassoulzadegan 1990; First et al. 2012). They have various habitats like freely swimming in the water, crawling on surfaces, and physically attached to surfaces by very flexible spring-like stalk, e.g., *Paramecium*, *Euplotes*, and *Vorticella* (James

and Hall 1995). There are some ciliates, which have special cilia for swimming and hairs for predation known as membranelle, which help for catching massive bacteria or prey in food vacuoles. Ciliate feeding rates are very high; it was recorded that single ciliates can digest 1254 bacteria h^{-1} (Iriberri et al. 1995).

Flagellates are another member of protozoans bearing one or more flagella having a different size from 2 to 20 μm. They are versatile in nature like swimming freely or attaching to solid surfaces by trailing flagellum or stalks. Flagellates using these flagella either create feeding current or exploit it to put the water and prey in the oral furrow and at the base of the flagellum where the pseudopodia ingest the prey. Flagellates show selective grazing as per their size. They prefer smaller-size organisms as significant prey. It was reported that bacteria are more susceptible to flagellate grazing than other microbes having size >2.4 μm. Chrzanowski and Šimek (1990) reported that flagellate bacterial grazing rate varies from 2 to 300 bacteria h^{-1} (Davis and Sieburth 1984; Eccleston-Parry and Leadbeater 1994a).

Amoebas are widely occurring protozoans and are very normal in water, soil, and other habitats. They are abundant in the soil, i.e., 103–107 g^{-1} of dry soil, with varying size <10 μm. Amoebas play a very important function in the cycling of various minerals and minute supplements such as nitrogen and phosphorus, particularly in shallow levels of nutrient environments (Goldman et al. 1985; Eccleston-Parry and Leadbeater 1994b). Amoebae, ciliates, and flagellates together selectively nurture on bacteria and control bacterial soil population (Table 14.1). They act as an essential constituent of the "microbial loop" (Azam et al. 1983). They are well recognized as Rhizopoda amoebae because they use their cytoplasmic protrusions, i.e., pseudopodia, for locomotion and nourishment. Amoebae are of two types, naked amoebae and shelled amoebae (testate amoebae).

Naked amoebae have no perfect shape but show three major morphological forms, i.e., floating, active form with extended lobose; fan-shaped, slug-like pseudopodial form trophozoites; and smaller and dormant form called cyst, an unusual rounded form (Page 1988; Griffiths 1970). Typical examples of naked amoeba are *Amoeba*, *Acanthamoeba*, *Vannella*, and *Vampyrella.*

Testate amoebae secrete the siliceous shell around the body. These testate are species-specific architectures. The testate shell amoebae designate the nutritional category of the living environment. The aperture is at one side of a shell, which is used for feeding or catching of different preys (Jassey et al. 2012). The dominant victims of amoebae are bacteria; the intake rate of the amoebic cell was reported to be 0.2–1465 bacteria h^{-1} (Heaton et al. 2001; Huws et al. 2005).

14.4.2 Role of Protozoans as New Bioinputs

Various studies indicated that protozoans majorly preyed upon bacteria. Bacteria, unicellular fungi, yeast, algae, and cyanobacteria were assumed as a nutritional capsule. In addition to nitrogen and carbon sources, these nutritional capsules are enriched with micro- and macronutrients in addition to various growth factors (Table 14.2). It was formerly confirmed that the nitrogen and carbon content of a

Table 14.1 Bacterivorous capacity of various protozoans

Types	Example	Bacterivorous capacity (bacterial cell h^{-1})	References
Amoeba			
Naked	*Saccamoeba*	0.2–1465	Heaton et al. (2001) and Huws et al. (2005)
	Acanthamoeba		
	Euglypha cristata		
	Hartmannella		
	Cf. *Mayorella*		
	Cf. *Polychaos*		
	Vannella		
	Vampyrella		
Shelled	*Arcellinid testate*		
	Euglypha cristata		
	Arcella gibbosa		
	Difflugia		
	Foraminifera		
	Nebela		
Flagellates	*Giardia intestinalis*	2–300	Davis and Sieburth (1984) and Eccleston-Parry and Leadbeater (1994a)
	Peltomonas hanelisp. nov.		
	Apusomonas australiensis sp.		
	Cetcomonar crassicauda		
Ciliates	*Paramecium*	20–1254	Iriberri et al. (1995)
	Vorticella		
	Balantidium coli		
	Oxytricha trifallax		
	Stentor roeselii		

Table 14.2 Elemental composition of bacteria and fungi

Element	Bacteria (% dry weight)	Fungi (% dry weight)
Carbon	50–53	40–63
Hydrogen	7	–
Nitrogen	12–15	7–10
Phosphorus	2.0–3.0	0.4–4.5
Sulphur	0.2–1.0	0.1–0.5
Potassium	1.0–4.5	0.2–2.5
Sodium	0.5–1.0	0.02–0.5
Calcium	0.01–1.1	0.1–1.4
Magnesium	0.1–0.5	0.1–0.5
Chloride	0.5	–
Iron	0.02–0.2	0.1–0.2
References	Luria (1960)	Lilly (1965)
	Aiba et al. (1973)	Aiba et al. (1973)
	Herbert (1956)	

fungal and bacterial cell are 10–15% and 50–63% by dry weight of fungi and bacteria, respectively. Similarly, bacterial and fungal mass sufficiently contain valuable micronutrients such as phosphate, potassium, sulphur, calcium, and iron (Luria 1960; Herbert 1956; Aiba et al. 1973). All protozoans are well characterized for their enormous feeding habits on other microbes such as bacteria and other microbes. Different soil bacterial flora assimilated the atmospheric nitrogen with organic and inorganic matters from the soil and locked in their cells, which are not freely accessible for the plants. The enormous grazing activity remobilized this immobilized nitrogen and released ammonia, which is ultimately utilized by the plant (Goldman and Caron 1985). Griffith and Bardget (1997) proved that the nitrogen requirement of protozoans is comparatively less, and they make about 60% of ingested nitrogen available to plants in the form of ammonia. Hence after the ingestion of bacteria by a protozoan, nitrogen is not only released but also various nutrients like 50–63% carbon, 2.0–4.5% phosphorus, and 0.02–0.5% iron (Table 14.3). Bonkowski (2004) reported the essential function of protozoa in sustaining soil productiveness and plant health.

Protozoa provide all essential nutrients by mineralizing complex material in bacteria during feeding. They also control the structure and activity of bacterial loops of soil and root-associated communities (Sieburth and Davis 1982; Bonkowski and Brandt 2002). Krome et al. (2010) reported that selective predation of bacteria promotes the production of various plant growth hormones. Besides offering different mineralized nutrients, it was proved that protozoans also increased the nutrient assimilation rate by altering the root morphology. Bonkowski and Brandt (2002) reported that when the *Acanthamoeba castellanii* was inoculated in the rhizosphere, it induces the extensive fibrous and fine root, suggesting that protozoans play an important role like plant growth hormones (Krome et al. 2010). Jousset et al. (2010) also proved that protozoans not only stimulate growth but also play a noteworthy function in pathogen suppressions by encouraging other bacterial soil flora for antibiotics like chemicals. Similarly, it induces iron chelating organic molecule production, which makes iron unavailable for plant pathogen growth and multiplication (Levrat 1989; Mazzola et al. 2009; Müller et al. 2013; Mellano et al. 1970).

Nielsen et al. (2002) proved that bacteria such as *Pseudomonas* and *Bacillus* produce various antipathogenic compounds such as phenazines, DAPG (diacetyl phloroglucinol), and cyclic lipopeptides like tensin, amphisin, and viscosinamide, but Mazzola et al. (2009), Jousset and Bonkowski (2010), and Weidner et al. (2017) revealed that protozoan grazing pressure induced the making of such antipathogenic

Table 14.3 Performance of protozoans for phosphatases, ACCD, and tryptophan

Sr. no.	Bacterivorous organism	Phosphatase (IU/h)	ACC deaminase activity (μM of α-ketoglutarate/mg/h)	Tryptophan (μg/h)
1	*Acanthamoeba* sp.	16.20	0.161	15
2	*Paramecium* sp.	18.40	0.093	17
3	*Amoeba* sp.	11.20	0.218	11
4	*Tetrahymena* sp.	14.00	0.187	07

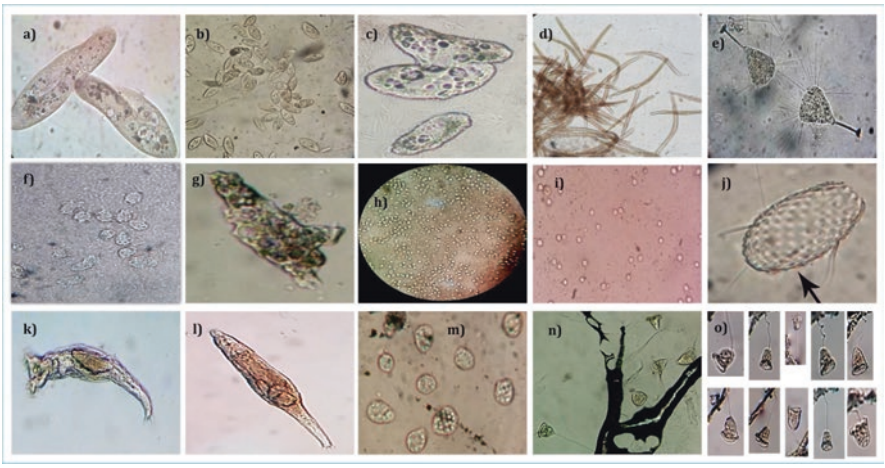

Fig. 14.8 Bacterivorous animals of soil cultured at School of Life Sciences, KBC NMU laboratory (**a–c**) *Paramecium* sp., (**d**) *Spirostomum* sp., (**e**) *Suctoria* sp., (**f**, **g**) *Acanthamoeba* sp., (**h**, **i**) cyst of amoebae, (**j**) testate amoebae, (**k**, **l**) Rotifer, (**m**) *Actinosphaerium* sp., (**n**, **o**) *Vorticella* sp.

fungal and bacterial compound. Recently in our laboratory studies at KBC North Maharashtra University (KBC NMU), Jalgaon, we have isolated and cultured various important agricultural bacterivorous animals, viz., *Paramecium*, *Amoeba*, Rotifer, and Vorticella (Fig. 14.8). It was revealed that *Acanthamoeba castellanii*, *Paramecium caudatum*, *Spirostomum*, and *Amoeba* spp. have the potential to produce various enzymes like phytase, phosphatase, and ACC deaminase. All these enzymes previously assumed the essential character of plant growth–promoting bacteria (Zahir et al. 2004). In laboratory-grown culture studies, it was discovered that *Paramecium* and *Acanthamoeba* efficiently utilized ACC and phytate and phosphate. Similarly, *Suctoria* sp. and *Spirostomum* were also investigated to use phosphate, phytic acid, and ACC like substrate at low concentrations (Table 14.3). *Amoeba* sp., *Acanthamoeba,* and *Paramecium* sp. were also found to be the producer of metabolic products such as amino acids like tryptophan, which was previously reported for a vital role in the stimulation of auxin production (Krome et al. 2010).

Sayre (1973) reported the potential of *Amoebae* as a future potent nematicidal agent. At KBC NMU laboratory, the cultured *Amoebae* sp. was also established to have an extraordinary potential of controlling invasive plant nematodes. Nematodes are the root-knot disease-causing agents of tomato and brinjal, i.e., *Meloidogyne incognita* and *Meloidogyne javanica.* It was observed that amoeba had 50–65 egg ingestion rate per amoeba per 24 h of both *Meloidogyne incognita* and *Meloidogyne javanica* and the 10–20 juvenile and 6–7 adult nematode ingestion per amoeba in 24 h.

14.5 Conclusion

Currently, nitrogen fixers, phosphate solubilizers, mycorrhiza, and biocontrolling agents like *Trichoderma* sp. are the most popular bioinputs throughout the world, even though it is necessary to recommend the utilization of other microbial bioinputs like ACCD, phytase producing microorganisms, Zn, K, S mobilizers. Besides that, latest studies proved the extraordinary potential of protozoa as the real new age bioinput, which proved their beneficial power for plant growth development, soil fertility augmentation, and biocontrol of soilborne pathogen. Recent advances in protozoans as bioinput will open a new avenue for plant–microorganism interaction research to solve current agricultural problems. The microbes present in the soil employ different strategies, and these beneficial belowground microbial interventions influence the plant beneficially. The character of these new age agricultural bioinputs is noteworthy for soil and plant well-being through nutrient fixation, solubilization, mineralization, and mobilization that are eventually accountable in the agroecological perspective. Such modern biological inputs in agriculture will help to achieve the future food demand of a growing world population and address the global problem of food security and malnutrition. So there is much more to do with nature's gift microorganisms which have tremendous metabolic flexibility and potential functionality.

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