CHAPTER

15

Diversity and Applications of *Penicillium* spp. in Plant-Growth Promotion

Mohd M. Altaf[1], *Mohd Imran*[2], *Hussein H. Abulreesh*[3], *Mohd S.A. Khan*[4] *and Iqbal Ahmad*[1]

[1]Aligarh Muslim University, Aligarh, Uttar Pradesh, India [2]Integral University, Lucknow, Uttar Pradesh, India
[3]Umm Al-Qura University, Makkah, Saudi Arabia [4]Imam Abdulrahman Al Faisal University, Dammam, Saudi Arabia

15.1 INTRODUCTION

The global necessity to increase agricultural production from a steadily decreasing and degrading land resource base has placed considerable strain on our fragile agro-ecosystem. The chemical oriented agriculture,a component of our "green revolution" policy, has reduced soil fertility significantly in extensive agricultural structure. This is demonstrated by decreased crop productivity from the same piece of land, soil deterioration, extensive green house gas discharge, build-up of pesticides and chemical fertilizers, groundwater contamination, and decline in ground water level because of unnecessary irrigation (Tilman et al., 2002; Foley et al., 2011). Soil is a dynamic and living entity that has remarkable flexibility to bounce back and restore majority of its ecological functions after being distributed anthropogenic interventions (Raman, 2005). Soil is a critical resource not only for agricultural production and food security but also for maintenance of most life processes and nutrient cycling. There is thus an urgent demand/need to adopt strategies to minimize the deterioration of soil health, improve productivity, and to reduce environmental hazards.

Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which mostly rely on soil biological processes and soil biodiversity. The complex soil biodiversity reflects a great variability among the living entities in the soil ranging from the myriad of invisible microbes, bacteria, fungi protozoan, and nematodes to familiar macrofauna such as earthworms and termites (Singh and Singh, 2008). Microorganisms in soil are critical for the maintenance of soil function in both cultivated and uncultivated soils because they play an important role in soil structure formation, decomposition of organic matter, toxin removal, and cycling of essential elements for plant growth (carbon, nitrogen, phosphorus, sulfur and other elements) (Van Elsas and Trevors, 1997). Soil microbial communities are often difficult to characterize, mainly because of their immense phenotypic, genotypic diversity, and heterogeneity. Bacterial populations in soil top layers can go up to more than 10^9 cells g^{-1} soil (Torsvik and Ovreas, 2002). Most of these cells are unculturable. The assistance of microorganisms to soil organic matter has not been recognized or considered unimportant, as the active microbial biomass in soils normally comprises less than 5% of the total organic matter in soils (Wardle, 1992; Branco de Freitas Maia et al., 2013). The soil environment is supposed to consists of great diversity of fungal species. Current knowledge of fungal diversity in soil is based largely on observations of the fruiting bodies present in an environment or from cultures obtained from soil isolation exercises. Only 17% of soil fungi are culturable (Bridge and Spooner, 2001; O'Brien et al., 2005).

Fungi are eukaryotic organisms and form a separate kingdom from plants and animals. Phyla ascomycota and basidiomycota comprise about 80% of all described fungal species and are either single-celled organisms growing as yeast, or multicellular organisms growing as filamentous hyphae (Berbee and Taylor, 1999). The hyphal growth

New and Future Developments in Microbial Biotechnology and Bioengineering.
DOI: http://dx.doi.org/10.1016/B978-0-444-63501-3.00015-6

mode of filamentous fungi is well adapted to find and exploit nutrient sources in the highly heterogeneous soil environment (Robson, 1999). Filamentous fungi have the ability to adopt both explorative and exploitative growth strategies and form linear organs of aggregated hyphae for protected fungal translocation (Fomina et al., 2005a, 2005b). Some fungi are polymorphic, occurring as both filamentous mycelium and unicellular yeasts or yeast-like cells, as in black meristematic or microcolonial fungi colonizing rocks (Gorbushina et al., 1993; Gorbushina, 2007). Fungi can also grow inside their own parental hyphae, utilizing dead parts of the colony under the protection of parental cell walls (Gorbushina et al., 2003). The capability of fungi to translocate nutrients through the mycelial network is important for exploring heterogeneous environments (Boswell et al., 2003, 2006).

Fungi are chemoheterotrophic organisms ubiquitous in nature in subaerial and subsoil environments, and considered as important decomposers and mutualistic symbionts of animal and plants. They are pathogenic and involved in the spoilage of natural and manufactured materials (Gadd, 2007). Fungi also have an important role in the maintenance of soil structure, due to their filamentous branching growth habit and frequent exopolymer production. A fungal role in biogeochemical cycling of the elements (e.g., carbon, nitrogen, phosphorus, sulphur, metals) is obvious and interlinked with the ability to adopt a variety of growth, metabolic, and morphological strategies, their adaptive capabilities to environmental extremes, and their mutualistic associations with animals, plants, algae, and cyanobacteria (Gadd and John, 2010). In aerobic environments they are of great importance, especially when considering rock surfaces, soil, and the plant root-soil interface (Gadd et al., 2007; Gadd, 2010). For example, mycorrhizal fungi are found to connect with 80% of plant species, and are involved in major mineral transformations and redistributions of inorganic nutrients, such as essential metals and phosphate, as well as carbon flow (Smith and Read, 2008). Free-living fungi also play major roles in the decomposition of plant and other organic materials like cellulose, lignin, chitin, etc., including xenobiotics, as well as mineral solubilization (Gadd, 2004).

Recent development of molecular techniques for identification and exploration of nonculturable soil fungi has provided much needed information. However, both culturable and nonculturable approaches have their own advantages and limitations (Garbeva et al., 2004). In recent years molecular techniques have been used by many workers to study soil microbial composition and diversity (Zheng-ji et al., 2008; Rincon-Florez et al., 2013; Lagos et al., 2015).

Microbial activity in the rhizosphere affects rooting patterns and the supply of available nutrients to plants, thereby modifying the quality and quantity of root exudates (Bowen and Rovira, 1999; Barea, 2000). Beneficial saprophytes from different of microbial groups help promote plant growth and health (Barea et al., 2004). Plant-growth promoting (PGP) properties of mycorrhizal fungi are well documented in phosphorus-deficient soil or soil poor in organic phosphorus. Among free-living soil fungi phosphate-solubilizing fungi such as *Aspergillus* and *Penicillium* are known to enhance plant growth and yield by providing available phosphorus and other plant-growth hormones such as IAA (indole 3- acetic acid) and gibberellins etc. Such fungi also synergistically interact with nitrogen-fixing bacteria in legume crops (Gaur, 1990; Abd-Alla et al., 2014; Bhuyan et al., 2015). In this chapter we review the diversity, characteristics for identification, and PGP mechanisms of the genus *Penicillium* and its impact on crop productivity as a bioinoculant.

15.2 *PENICILLIUM* AND ITS TAXONOMICAL BACKGROUND

Penicillium is a well-recognized and widely distributed fungi present in different environments. It is universally present and has a tremendous economic impact on human life. Basically, it works as a decomposer of organic substances present in nature, whereas some of the species of *Penicillium* create lot of nuisance in the form of rotting of food crops (Frisvad and Samson, 2004; Samson et al.,2010), as well as secreting different varieties of mycotoxins (Frisvad et al., 2004). On the other hand, other strains act as beneficial organisms; for example, in the production of special cheeses, such as Camembert or Roquefort (Giraud et al., 2010) and fermented sausages (Lopez-Díaz et al., 2001; Ludemann et al., 2010). This genus has also been screened for the production of different enzymes due to its decomposing potential (Crueger and Crueger, 1990; Li et al., 2007; Terrasan et al., 2010). The significance of this genus became famous with the production of penicillin, which transformed medical approaches to treating bacterial diseases.

Several workers proposed different methods for the isolation and identification of genus *Penicillium* (Houbraken and Samson, 2011; Visagie et al., 2014). *Penicillium* can be identified by the secretion of a typical reproductive makeup called “penicillius” (meaning little brush). With over 200 known species and omnipresent

distribution in soil, Penicillia are considered as one of the main types of fungi and among the most universal eukaryotic lifeforms on earth (Pitt et al., 2000). Penicillia are known as blue and green molds that develop on citrus (*P. digitatum* and *P. italicum*) and as the growing agent of various cheeses and meats (e.g., *P. roqueforti var. roqueforti* and *P. camemberti*) (Geisen et al., 2001). Alexander Fleming (1929) made the genus famous through the discovery of the antibiotic "penicillin" from a culture of *P. chrysogenum* (*P. notatum*), noting its ability to inhibit the growth of the pathogenic bacterium *Staphylococcus aureus* (Raper and Thom, 1949). It has now been more than 200 years since Link (1809) established the generic name *Penicillium* and described the three species: *P. candidum, P. glaucum,* and the generic type *P. expansum*. Since then, more than 1000 names have been included in the genus. At present several of these names are not identifiable because descriptions are not considered complete by modern standards. A number of names were published untenably, or are now considered synonyms of other species. Thom (1930) corrected all species illustrated until 1930 and accepted 300 species. In later studies, Raper and Thom (1949) accepted 137 species, Pitt (1979) accepted 150 species, and Ramírez (1982) accepted 252 species (numbers include species described in *Eupenicillium*). During that period, a morphological species theory was employed for *Penicillium* classification and identification, and later DNA sequencing began to be used in the 1990s. DNA sequencing produced the risk for old names of fungi previously considered for uncertain applications (because their ex-type cultures were no longer morphologically representative), could replace more commonly used but younger names. As such, the List of "Names in Current Use" (NCU) for the family *Trichocomaceae* (Pitt and Samson, 1993) included 223 species and disregarded all other names as if not published. This catalog was modernized by Pitt et al. (2000) who accepted 225 species. Species names not accepted on these lists should not be disregarded permanently, as argued by Pitt et al. (2000), because they were not formally rejected under the nomenclatural code and could still be reintroduced in a revised taxonomy. In fact, this became universal practice as numerous old species discovered were found to be different and were reintroduced (Peterson et al., 2005; Serra et al., 2008; Houbraken et al., 2012; Visagie et al., 2013).

The rejection of article 59 in the new International Code of Nomenclature for algae, fungi, and plants (ICN) (McNeill et al., 2012) resulted in single-name nomenclature for fungi. In expectation of this change, Houbraken and Samson (2011) redefined the genera in the family *Trichocomaceae* based on a four-gene phylogeny. They separated the *Trichocomaceae* into three families: the Aspergillaceae (*Aspergillus, Hamigera, Leiothecium, Monascus, Penicilliopsis, Penicillium, Phialomyces, Sclerocleista, Warcupiella, Xeromyces), Thermoascaceae (Byssochlamys/Paecilomyces, Thermoascus*) and the Trichocomaceae *(Rasamsonia, Sagenomella, Talaromyces, Thermomyces, Trichocoma*). *Penicillium* subgenus *Biverticillium* and *Talaromyces* were found to create a monophyletic clade different from other subgenera of *Penicillium*, and these names were recombined as necessary into *Talaromyces* (Samson et al., 2011). The left behind *Penicillium* species created a monophyletic clade together with species classified in *Eupenicillium, Eladia, Hemicarpenteles, Torulomyces, Thysanophora,* and *Chromocleista.* These generic names became synonyms for *Penicillium*, and their species were given *Penicillium* names. The remaining three *Aspergillus* species, *A. paradoxus* (*Hemicarpenteles paradoxus*), *A. malodoratus,* and *A. crystallinus*, phylogenetically belonging to *Penicillium*, were transferred to *Penicillium*. In order to contain the morphological dissimilarity, the generic analysis of *Penicillium* was adjusted by Houbraken and Samson (2011). Significantly, in contrast with the prevailing generic idea, it now eliminated the acerose phialides and usually symmetrically branched conidiophores of species now included in Talaromyces, and was expanded to include the conidiophores with solitary phialides of species in section Torulomyces, and the darkly pigmented stipes that formerly characterized the genus Thysanophora, which show secondary growth by means of the proliferation of an apical penicillus. For infrageneric classification, the genus was divided into two subgenera, *Aspergilloides* and *Penicillium*, with 25 sections (Visagie et al., 2014**).**

Thom (1954) defined the idea of species employed for *Penicillium*. He was the pioneer of standardized working techniques and stressed that *Penicillium* taxonomy demands a consistent, logical approach. He confirmed these tendencies himself by taking into account infraspecies differences when outlining species. To reduce infraspecies/intraspecies dissimilarity, the importance of standardized working techniques was again highlighted by Pitt (1979), Samson and Pitt (1985), Okuda (1994), and Okuda et al. (2000). Comparing strains by means of morphology requires experience and nuance, because of the deterioration of characters in old reference material and the large number of species in the genus. New techniques include taxonomic studies resulting in the physiological species concept (Ciegler and Pitt, 1970; Pitt, 1973; El-Banna et al., 1987; Paterson et al., 1989), phylogenetic species idea, together with Genealogical Concordance Phylogenetic Species Recognition (GCPSR) (LoBuglio et al., 1993; Berbee et al., 1995; Skouboe et al., 1999; Taylor et al., 2000), and finally directed to the joint approach using morphological, extrolite and genetic data in a polyphasic species concept (Christensen et al., 2000; Frisvad and Samson, 2004). However, in current taxonomy, sequence data and GCPSR hold extra burden than morphology or extrolite data. Still *Penicillium* species are universal and the taxonomic structure of the genus is definite, species

identification is still difficult. Troubles comprise an obsolete traditional species list and a lack of a confirmed, complete sequence database.

15.2.1 Characteristics of *Penicillium*

Morphology was fundamental to the taxonomy of *Penicillium* and together with multigene phylogenetics and extrolite outlining includes the polyphasic species model adopted for *Penicillium*. Morphology is defined as the bodily structural design, which is used as a model that works as organisms and acclimatize to its surroundings, but few approaches can be dissimilar or may be introduced by species signals in their surrounding environment (Visagie et al., 2014). Therefore strains distinguished in one laboratory may appear dissimilar when cultured in other because of substantial differences in nutrients, temperature, lighting, or humidity. This problem occasionally makes evaluation among diverse studies difficult. These problems/influences might be mitigated by employing universal working methods for medium preparation, inoculation technique, and incubation conditions (Samson and Pitt, 1985; Okuda, 1994; Okuda et al., 2000).

15.2.2 Cultural characteristics

Among fungi species recognition are based up to certain extent on the colony characteristics and diameters on specific media. Czapek Yeast Autolysate agar (CYA) and Malt Extract agar (MEA) is suggested as standard media for *Penicillium* (Visagie et al., 2014). In current taxonomic analysis two diverse MEA compositions are extensively employed. Blakeslee's MEA is traditionally used. Both are appropriate for description, but analysis should clearly note which composition of MEA was used. Other media are in use for studying taxonomic characteristics such as Czapek's agar (CZ), yeast extract sucrose agar (YES), oatmeal agar (OA), creatinesucrose agar (CREA), dichloran 18% glycerol agar (DG18), Blakeslee's MEA, and CYA with 5% NaCl (CYAS) (Visagie et al., 2014). CZ agar is used for extrolite profiling of species. Sexual reproduction mostly occurs when strains are cultured on OA and hence frequently give valuable taxonomic information. OA should be prepared using organic uncooked flakes and not the unsuitable quickcook oats or prefabricated OA formulations available. Acid production is observed by color reaction in CREA (from purple to yellow) and is often useful for distinguishing between closely related species. In some species, acid production is followed by base production. DG18 and CYAS often give valuable data with respect to growth rates on low water activity media. For regular conidial colors, the addition of zinc-sulphate and copper-sulphate as trace elements (1 g $ZnSO_4.7H_2O$ and 0.5 g $CuSO_4.5H_2O$ in 100 ml distilled water) is significant as these metals differ in water sources in different areas and are important for pigment production.

For inorganic chemicals, analytical grade should be employed. Practice has revealed that the agar brand significantly affects the colony phenotype. As such, it is important to test the agar for consistent character development and note the brand in species descriptions. Media are prepared in 90 mm Petri dishes with a volume of 20 mL (Okuda et al., 2000). Glass Petri dishes were once considered the best for character observation. However, today it is not feasible to use them, and polystyrene Petri dishes are recommended. The Petri dishes should preferably be vented to allow for a bit of air exchange. Inoculations are made from spore suspensions in a semisolid agar solution containing 0.2% agar and 0.05% Tween80 (Pitt, 1979). In some laboratories, spore suspensions are made in a 30% glycerol, 0.05% agar, and 0.05% Tween80 solution. Variation between these two inoculation suspension solutions has not been shown. Visagie et al. (2014) advocated for the use of micropipette for inoculating spore suspension in three-point fashion (0.5–1 μL per spot). Wrapping with Parafilm must be avoided, since it hampers air transfer and reduces growth and sporulation (Okuda et al., 2000). In the case of walk-in incubators, the plates should be placed either in plastic bags or plastic containers with enough aeration without overcrowding. All media should be incubated at the standard temperature of 25°C for 7 days, with additional CYA plates at 30°C and 37°C to help differentiate among species. Temperatures should also be monitored routinely as slight differences can create huge impacts on colony growth and development. After the targeted incubation period (7 days), the colony characteristics are measured including colony diameter, colony texture, degree of sporulation, the color of conidia, the abundance, texture and color of mycelia, the presence and colors of soluble pigments and exudates, colony reverse colors, and degree of growth and acid production (in some species acid production followed by base production) on CREA. The utilization of a standard color chart is suggested for descriptions. The widely used color chart for *Penicillium* is the *Methuen Handbook of Color* (Kornerup and Wanscher, 1967), which although long out of print is available in many libraries. It is significance to note that color names

used by Raper and Thom (1949) were founded on Ridgeway (1912) and are also still in use. When color charts are not obtainable, it is suggested to publish full color photo-plates to convey descriptions of new species (Visagie et al., 2014).

15.2.3 Microscopic Structural Characteristics

Conidiophore and cleistothecium are key factors of *Penicillium* that play a significant role in taxonomic description. The conidiophore branching profile has been conventionally employed in the classification of *Penicillium* (Pitt, 1979). The conidiophores vary from being simple (solitary phialides) to very complex patterns with several stages of branching ensuing in overall symmetrical or asymmetrical patterns. Monoverticillate conidiophores have a terminal whorl of phialides and in some species, the terminal cell of the conidiophore is slightly swollen or vesiculate. Such species might be puzzled with diminutive *Aspergillus* conidiophores, but they have septa in the stipes unlike species of the latter genus. Divaricate conidiophores, previously also referred to as irregular, are best described as having a simple to complex branching pattern with numerous subterminal branches, but where conidiophores parts are divergent. Biverticillate conidiophores have a whorl of three or more metulae between the end of the stipe and the phialides; the metulae may be of unequal or equal length, vary in their degree of divergence, and are usually more or less cylindrical but can also be clavate or slightly vesiculate. Terverticillate conidiophores have is another level of branching between the stipe and the metulae, often just a continuation of the stipe axis and one side branch, sometimes a true whorl of three or more branches. Quaterverticillate conidiophores are produced by only a few species, and have one extra level of branching beyond the terverticillate pattern. Terverticillate and quaterverticillate conidiophores tend to be conspicuously asymmetrical. In colonies of many species, especially as cultures begin to degenerate, there may be more than one branching pattern or intermediate form, and it can be challenging to decide which pattern is typical or most developed (Visagie et al., 2014). Other significant microscopic characteristics consist of the wall texture/ornamentation of stipes and conidia, as well as dimensions, ornamentation, and sometimes colors of all elements of the conidiophores. Wall textures are considered to be highly susceptible to minute differences in media composition and aeration. For best observation of conidial ornamentation, differential interference contrast (Nomarski) is recommended if possible; ornamentation is sometimes most conspicuously visible in air pockets in the preparation. In the microscopic studies of *Penicillium*, slides are prepared from 7–10 day old MEA colonies. To examine conidia, matter detached from colony centers generally gives more uniform results. Lactic acid (60%) is widely used as a mounting fluid, while other solutions such as Shear's solution or lactic acid with cotton blue can also be applied (Frisvad and Samson, 2004; Samson et al., 2010).

Lactophenol is not normally suggested because of the corrosiveness and toxicity of phenol. Because of the plentiful hydrophobicconidia created by most species, drops of 70% ethanol are normally used to wash away excess conidia and to prevent air from being "trapped" when mounted in lactic acid. For photographing conidiophores, we often wash away the spores two or three times. Some species have dense colonies, and sometimes it is necessary to tease apart the conidiophores with very fine needles under the dissecting microscope (Visagie et al., 2014).

15.2.4 Molecular Taxonomy: DNA Barcoding

During the 1990s, DNA sequencing generated new interest in understanding associations among species without the necessity of standardizing the culturing regimes and eradicating the troubles associated with distorted cultures. It also created new avenues for sequence-based identifications. DNA barcoding was initiated to identify species of any eukaryotic organism by using a standardized short DNA sequence and accurate reference database linked to authoritatively identified vouchers (Blaxter et al., 2005; Seifert et al., 2007; Schoch et al., 2012). However, in recent times, internal transcribed spacer rDNA area (ITS) has been acknowledged as the authorized barcode for fungi. ITS is the most widely sequenced marker for fungi, and universal primers are available (Schoch et al., 2012). For *Penicillium* and many other genera of ascomycetes, ITS is not variable enough for characterizing all closely related species (Seifert et al., 2007; Schoch et al., 2012). The open-source sequence repository GenBank contains a large proportion of incorrectly identified sequences, making identification of *Penicillium* using BLAST very tricky for inexperienced workers (Santamaria et al., 2012; Koljalg et al., 2013; Schoch et al., 2014). For *Penicillium*, the International Commission of *Penicillium* and *Aspergillus* (ICPA), in combination with the publication of established species list, decided to include GenBank accession numbers to reference barcode sequences for

each species when available. Due to the restrictions associated with ITS as a species marker in *Penicillium*, a resultant barcode or identification marker is frequently desired for identifying isolates to species level. The necessities related to secondary identification marker are comprehensible. It must be trouble-free to amplify, distinguish between closely associated species, and lastly, the reference data set should be complete, signifying that there should be representative sequences for all species (Visagie et al., 2014).

Another tool that can be used as a secondary identification marker for *Penicillium* is β-tubulin (BenA). BenA is well suited for species identification in *Penicillium*. *Penicillium chrysogenum* and *Penicillium allii-sativi* is one case where care should be taken when detection is based on BenA (Houbraken et al., 2012). *Penicillium kongii* in recent times launched as a close relative of *Penicillium brevicompactum* (Wang and Wang, 2013). Although this species created a coherent clade different from the *P. brevicompactum* ex-type strain, on studying extra strains previously identified as *P. brevicompactum*, the *P. kongii* clade was determined to exist within the *P. brevicompactum* clade. This clade thus needs more data to separate species from the complex. A comparable situation has been seen detected for *Penicillium desertorum* and the newly described *Penicillium glycyrrhizacola* (Chen et al., 2013), where more strains and additional genes included in the phylogeny help to solve species. Some other species that cannot be recognized on a molecular basis include *Penicillium camemberti*, *Penicillium caseifulvum*, and *Penicillium commune*, which share identical BenA and other gene sequences (Giraud et al., 2010). Primers employed for amplification of ITS, BenA, and other genes are described by Visagie et al. (2014).

15.3 BIOCHEMICAL ACTIVITIES OF SOIL FUNGI RELEVANT TO PGP AND *PENICILLIUM*

Soil microorganisms are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in key processes such as soil structure formation, decomposition of organic matter, toxin removal, and cycling of carbon, nitrogen, phosphorus, and sulfur. In addition soil, microorganisms play key role in suppressing soilborne plant diseases and in promoting plant growth (Garbeva et al., 2004). The importance and role of soil fungi in PGP and soil health are well known. They produce a large number of secondary metabolites (IAA, siderophores, ammonia, organic acids, antibiotics, extracellular enzymes, etc.) and enhance plant growth, crop productivity, and soil fertility (Khan et al., 2010; Saldajeno and Hyakumachi, 2011). However, such studies are limited to a certain group of fungi such as mycorrizae and a few free-living phosphate-solubilizing fungi (Gaur, 1990; Khan et al., 2007). Some of the activities relevant to PGP are briefly described as follows.

15.3.1 Phosphate Solubilization

Phosphorus is one of the major nutrients (second only to nitrogen) in plants. A greater part of soil phosphorus, approximately 95%–99%, is available as insoluble phosphate and its bioavailability is negligible for plants (Vassileva et al., 1998). To increase the availability of phosphorus for plants, large amounts of fertilizer are applied to soil (Omar, 1998). Therefore little of the applied phosphorus is available to plants, making continuous application necessary (AbdAlla, 1994). However, phosphorus deficiencies are widespread in soil throughout the world and phosphorus fertilizers represent a major cost for agricultural production. Many soil fungi and bacteria are known to solubilize inorganic phosphates. Phosphate-solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to plants, allowing sustainable use of phosphate fertilizers. Application of PSMs in the field has been reported to increase crop yield. Several mechanisms like lowering of pH by acid production, ion chelation, and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSMs (Goldstein, 1986; Halder et al., 1991; AbdAlla, 1994; Whitelaw, 2000). Among PSMs, fungi perform better in acidic soil condition. Species of *Aspergillus*, *Penicillium*, and yeast have been widely reported to solubilize various forms of inorganic phosphates (Asea et al., 1988; Whitelaw, 2000). Fungi have been reported to have a greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996), because P-solubilizing fungi secrete more acids compared to bacteria and as a result exhibit better P-solubilization (Venkateswarlu et al., 1984). In India, it is estimated that about 260 million tons of phosphate rock deposits are available and this material may be a cheap source of phosphate fertilizer for crop production (FAI, 2002). Soil microorganisms that solubilize mineral phosphates can significantly affect phosphorus cycling in both natural and agricultural ecosystems. Phosphorus absorption by plants can be increased by the presence of symbiotic

organisms such as mycorrhizal fungi (Azcon-Aguilar et al., 1986) or by inoculation with the soil phosphate-solubilizing fungi (particularly black *Aspergilli*) (Vassileva et al., 1998; Reddy et al., 2002) and some species of *Penicillium* (Asea et al., 1988; Cunningham and Kuiack, 1992; Whitelaw et al., 1999) assimilated only as soluble phosphate. The contribution of mycorrizal fungi in phosphate mobilization is well known. However, in recent years many free-living fungi including *Penicillium* have been found to be promising agents to solubilize phosphate. Cunningham and Kuiack (1992) reported an isolate of *Penicillium bilaii* that can solubilize mineral phosphates. Wahid and Mehana (2002) reported about the three phosphate solubilizer fungal isolates, *Aspergillus niger*, *Aspergillus fumigatus*, and *Penicillium pinophilum*, isolated from the rhizosphere of different plants grown in Ismailia and South Sinai Governorates. The fungal isolates effectively solubilized rock phosphate or tricalcium phosphate in Pikovskaya's liquid medium. In pot and column experiments, they significantly reduced pH and increased available phosphorus in the soil treated with either rock phosphate or superphosphate. Plant uptake of phosphate was studied by Gupta et al. (2007). They found phosphate-solubilization properties among fungi and bacteria obtained from the heavy metal mines of Orissa (India). They screened 62 fungi and 253 bacteria for phosphate-solubilization properties, and 12 fungi and 19 bacteria were found to solubilize tricalcium phosphate (TCP). *Penicillium* sp. 21 solubilized and released 81.48 mg/mL whereas *Penicillium* sp. 2 showed better efficiency of rock phosphate solubilization and produced 4.87 mg/mL in the liquid culture. Bacterial strains were comparatively poor solubilizers of TCP and rock phosphate in solid and liquid culture. Phosphate-solubilizing fungi were acid producers and more efficient than bacterial isolates. *Penicillium* sp. 21 and *Penicillium* sp. 2 were confirmed to be the best for TCP and rock-phosphate solubilization. Vyas et al. (2007) reported *Eupenicillium parvum* as a phosphate-solubilizing fungal strain from tea rhizosphere and studied its qualitative and quantitative capability of phosphate solubilization in vitro. The fungus developed a phosphate-solubilization zone on modified Pikovskaya agar, supplemented with tricalcium phosphate. Quantitative estimation of phosphate solubilization in Pikovskaya broth showed high solubilization of tricalcium phosphate and aluminum phosphate.

Ahuja et al. (2007) tested a phosphate-solubilizing fungus, *Paecilomyces marquandii* AA1, from phosphate deficient soil on Pikovskaya's medium buffered with Tris-HCl pH 8. The organism could release phosphate from both buffered and unbuffered medium and solubilized rock phosphates from various places. Wakelin et al. (2004) reported on the phosphate-solubilizing fungi *Penicillium radicum*, *Penicillium bilaiae* (strain RS7B-SD1), and an unidentified *Penicillium* sp. designated strain KC6-W2 for their ability to increase the growth and phosphorus (P) nutrition of wheat, medic, and lentil in three soils of neutral to alkaline pH reaction. *Penicillium* sp. KC6-W2 was found to have the strongest PGP strain, stimulating significant increases in shoot growth and dry mass in seven of the nine experiments conducted. In an investigation of soil fungi from the Aligarh region of northern India (Imran, 2009) reported various filamentous fungi recovered from agricultural soils solubilizing phosphate to varying degrees. Similar reports are available from different parts of the world (Chai et al., 2011; De Oliveira Mendes et al., 2014; Yin et al., 2015). Different mechanisms implicated in PGP by phosphate-solubilizing soil fungi are briefly summarized in Fig. 15.1.

15.3.2 Indole Acetic Acid Production

Growth hormones are organic substances produced naturally in higher plants, controlling growth or physiological functions and able to mediate intercellular communications in minute amounts. Five major types of hormones regulate plant development: Auxin, Gibberellin, Cytokinin, Ethylene, and Abscisic acid. IAA is found universally and is a major plant hormone. It is one of the most common and extensively used auxin (Wilkins et al., 1972) in modern biotechnological methods. For example, in tissue culturing. IAA stimulates differentiation of root/shoot in an undifferentiated callus. Indole-3-acetic acid and its analogue is the primary active auxin in most plants. It is synthesized from tryptophan, primarily in leaf primodion and young leaves and in developing seeds. More than one pathway of IAA synthesis in plants has been demonstrated. Auxin plays an important role in cell enlargement, cell division, root initiation, etc. (Glick, 1995; Fu et al., 2015). Many fungi can produce auxins in axenic cultures (Gruen, 1959; Buckley and Pugh, 1971). Most species use tryptophan to produce indole-3-acetic acid (IAA), mainly through the indole-3-pyruvic acid and tryptamine pathways (Tudzynski and Sharon, 2002; Fu et al., 2015). The physiological role of auxins in fungi is not well understood. In most studies, auxin was added to the culture medium, and the effects on fungal growth and development were determined. It is not known whether exogenous IAA and endogenous IAA cause the same phenotype. One of the roles suggested for fungus-produced IAA is to mediate fungal-plant interaction (Fu et al., 2015).

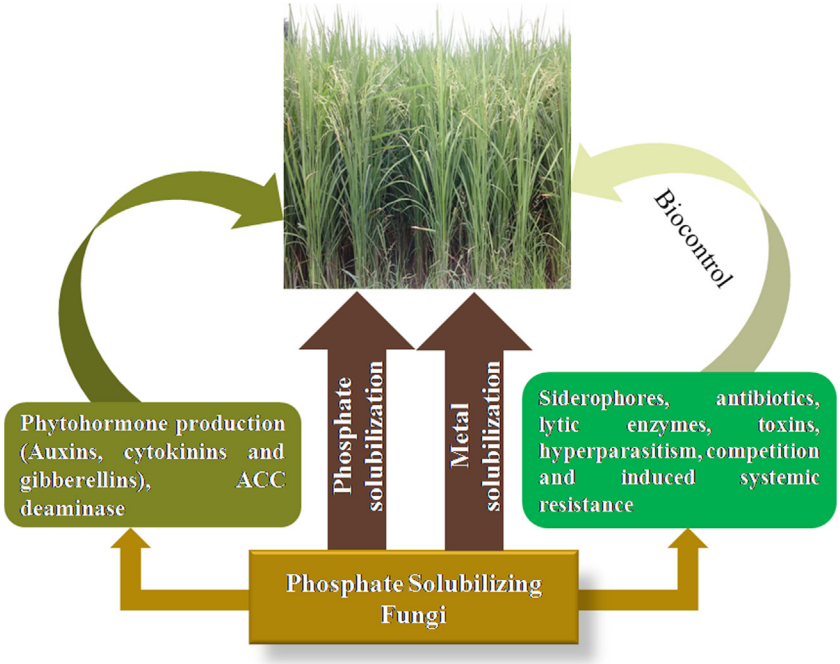

FIGURE 15.1 Mechanisms of plant growth promotion by phosphate solubilizing fungi.

Hasan (2002) screened fungi, mostly *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Penicillium corylophilum*, *Penicillium cyclopium*, *Penicillium funiculosum*, and *Rhizopus stolonifera*, for their ability to produce gibberellin and IAA. All fungal species have the ability to produce gibberellin (GA) but *F. oxysporum* was found to produce both GA and IAA. The optimum period for GA and IAA production by *F. oxysporum* was 10 days in the mycelium and 15 days in the filtrate at 28°C. Similar reports related to the production of these hormones by fungi, their role in plant-growth enhancement, and signaling during biotic and abiotic stresses has been reviewed by several researchers (Peleg et al., 2011; De Vleesschauwer et al., 2014; Pozo et al., 2015; Spence et al., 2015).

15.3.3 Siderophore Production

To sequester and solubilize ferric iron, many microorganisms utilize an efficient system consisting of low-molecular mass (<1000 Da) compounds with high iron affinity called "siderophores" (Neilands, 1995). According to the generally accepted definition, siderophores are ferric-specific microbial iron-chelator compounds whose biosynthesis is regulated by the availability of iron in the surrounding medium and under conditions of high iron concentration; the production of these compounds is repressed. Therefore a certain variation can be expected depending on the particular species under investigation (Neilands, 1984). Studies of microorganism siderophore producers have received much attention because of their clinical applications and potential utilization of these chelators in agriculture (Neilands, 1993, 1995; Saha et al., 2016). Most species of the genus *Aspergillus* are known to produce several hydroxamate-type siderophores and many reports on the isolation and characterization of siderophores have been published (Hider, 1984; Dube et al., 2000; Vala et al., 2006).

Some siderophores (e.g., aerobactin) have lower and others (e.g., enterobactin) have higher affinities. Siderophores are typically produced by bacteria, fungi, and monocotyledonous plants in response to iron stress (Ratledge and Dover, 2000). Typically, microbial siderophores are classified as catecholates, hydroxamates, and á-carboxylates, depending on the chemical nature of their coordination sites with iron (Winkelmann, 1991, 2002; Saha et al., 2016).

Hydroxamates are produced by fungi and bacteria, whereas catecholates are produced exclusively by bacteria and comprise catechol and hydroxy groups as ligands. á-carboxylates are produced by the group of fungal zygomycetes (mucorales) and a few bacteria, such as *Rhizobium* meliloti and *Staphylococcus hyicus*, and coordinate iron through hydroxy and carboxyl groups (Baakza et al., 2004). Although plentiful in the environment, iron exists in nature predominantly in the insoluble ferric iron oxidation state, which is not readily bioavailable for microbial

assimilation. Successful adaptation to this environmental context has provided almost all fungi with an iron uptake strategy that involves reduction of Fe^{3+} to Fe^{2+} via two general mechanisms: (1) iron reduction before uptake or reductive iron assimilation and (2) iron uptake before reduction or nonreductive iron assimilation (De Luca and Wood, 2000).

In brown-rot fungi, at least three strategies could be involved in this process. One mechanism is the secretion of low-molecular-weight Fe^{3+}-chelating hydroxamate siderophores. A second possible route to acquire iron could be the release of organic acids, as wood-decaying fungi are also known to produce oxalic and citric acids. The last iron acquirement approach may possibly based on discharge of catechols, connected with the extracellular nonenzymatic ferric-reducing activity (Arantes and Milagres, 2006).

Korat et al. (2001) examined 18 filamentous fungi belonging to *Zygomycotina* (10 mucorales) and *Ascomycotina* (4 *Aspergillus* spp. and 4 *Penicillum* spp.), and all produced siderophores in iron-deficient m9 and grimm-allen media except *Rhizopus* sp. and *Aspergillus flavus*. The siderophore produced by the 9 mucorales were carboxylates, while those produced by the 3 *aspergilli* and 4 *penicillia* were hydroxamates. They found the highest (72.2×10^{-4} g/mL) and lowest (14.9×10^{-4} g/mL^{-1}) siderophore producers to be *Rhizopus oryzae* and *Synchephalastrum* sp., respectively.

Baakza et al. (2004) examined the siderophore-producing potential of 20 fungal isolates (the same 10 species from each marine and terrestrial habitat) and compared them. Except marine *Aspergillus flavus*, all isolates produced siderophores as evidenced by positive reaction in $FeCl_3$ test, Chrome Azurol Sulphonate (CAS) assay, and CAS agar plate test. The results indicated widespread occurrence of siderophores in both habitats. They revealed that mucoraceous fungi produce carboxylate, while others produce hydroxamate siderophores. Among all the isolates, *Cunninghamella elegans* (marine form) was the highest siderophore producer (1987.5 Ag/mL) followed by the terrestrial form of *C. elegans* (1248.75 μg/mL). They observed that four terrestrial isolates (*A. niger*, *Aspergillus ochraceous*, *P. chrysogenum*, and *P. citrinum*) were ahead in siderophore production, while the other four were marine isolates. Perez-Miranda et al. (2007) screened about 48 microorganisms for siderophore production on popular CAS assay, based on the utilization of chrome azurol S. As a result, they determined 36 microorganisms were siderophore producers out of 48 isolates from three sampling sites.

15.3.4 Extracellular Enzymes

Enzymes are essential proteins for the metabolic system of all living organisms and have an important role in the degradation of organic matter, in host infection, and food spoilage. In the metabolic pathways, they act in organized sequences of catabolic and anabolic routes (Lehninger, 1988). Enzymes may also act in the control of biochemical processes in living cells. They may be isolated from plants, animals, and microorganisms. Microbes are considered good sources of industrial enzymes for the great diversity of enzymes that have been found (Lima et al., 1986). These enzymes are used in large scale in the textile (amylase, cellulose, oxidoreductase), food (pectinase, protease, cellulose, oxidoreductase), detergents (protease, lipase, cellulose, oxidoreductase), paper (xylanase, oxidoreductase and lipase), and leather (protease, lipase) industries (Nielsen and Oxenbøll, 1998).

Extracellular enzymes may be produced in liquid or solid media. The use of solid media permits fast screening of large populations of fungi, allowing the detection of specific enzymes and helping in the chemotaxonomical differentiation of many microorganisms (Alves et al., 2002). Filamentous fungi such as *A. niger*, *Aspergillus oryzae*, and *Trichoderma reesei* are able to produce and secrete large concentrations of enzymes (e.g., amylases, proteases, cellulases) into the environment. Efficient fermentation technologies have been developed for antibiotic, organic acid, and native enzyme production from filamentous fungi (Wiebe, 2003).

Soil microbial communities are important contributors to the decomposition of organic matter. Saprophytic fungi play a major role in decomposition because they must rely on dead organic matter as their source of carbon and energy. Mycorrhizal fungi, which obtain carbon primarily from their host plants, contribute to decomposition as they can access organic sources of nitrogen (Bending and Read, 1996). The chemical composition of soil organic matter is an important factor controlling the activity of soil microbes and decomposition processes (Waldrop et al., 2006).

Lignin is an energy-rich, recalcitrant polyphenolic macromolecule and its decomposition is accomplished in large part by the activity of extracellular enzymes that catalyze the oxidation of phenylpropane alcohols from the lignin polymer (Kirk and Farrell, 1987). Lab studies have found saprophytic fungi possess the physiological capacity to produce phenol oxidase and peroxidase, two enzymes important in lignin depolymerization (Colpaert and Laere, 1996; Courty et al., 2008). The inhibition of lignin degradation may be due to changes in the

composition or abundance of the soil fungal community, or it may be due to repressed fungal enzyme production or activity (Kirk and Farrell, 1987; Fog, 1988). α-Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (Pandey et al., 2000). Fungal sources are confined to terrestrial isolates, mostly to *Aspergillus* species and to only one species of *Penicillium*, *P. brunneum* (Pandey et al., 2000).

15.4 *PENICILLIUM* INOCULANT PRODUCTION AND UTILIZATION

The pioneering work of Gaur (1990) on phosphate-solubilizing microorganisms encouraged several workers to reinvestigate new PGP activities in free-living soil fungi (Khan et al., 2007; Babu et al., 2015). Phosphate-solubilizing fungi were developed as bioinoculants for various crop productions. The living microbial preparations with phosphate-solubilizing properties are known as microphos. The manufacture of microphos consists of three main steps: (1) selection and in vitro evaluation of P-solubilizing potentials of the fungal strains; (2) selection of carriers, amalgamation of inocula with preferred carriers, and suitable development of fungal inoculants; and (3) testing of the quality of inoculants in terms of persistence of P-solubilizing activity, viable fungal load g^{-1} of carrier, and proper delivery to farmers. Different carrier materials used for microphо production include soil, cow dung cake powder, peat, and farmyard manure (FYM) (Gaur, 1990; Khan et al., 2010). After their production, these bioinoculants are packed in polythene bags and stored for three months at 25°C ± 2°C. Likewise, the two cultures from the identical groups (fungi) or different groups (one or two fungi/AM fungi and one or more bacteria) can be mixed together in order to produce a mixed/coinoculant. However, before the use of different microorganisms, their compatibility toward each other and the presence of desirable PGP activity under in vitro conditions should be established. If the two organisms demonstrate any kind of antagonism under laboratory conditions, they should not be utilized collectively for preparing a mixed or coculture of the microphos. By employing this kind of plan, a microbial preparation called Indian Agricultural Research Institute (IARI) microphos culture was developed in India (Gaur, 1990), which consists of two efficient phosphate-solubilizing bacteria (*Pseudomonas striata* and *Bacillus polymyxa*) and three phosphate-solubilizing fungi (*Aspergillus awamori*, *A. niger*, and *Penicillium digitatum*). Moreover, Novozymes Biologicals Limited (Canada) has commercially released *Penicillium bilaii* as bioinoculant to help increase phosphate nutrition (JumpStart® LCO) and *P. radicum* (PR-70 RELEASE; BioCare Technology, Somersby, Australia).

Traditionally the most common and extensively used process for the application of bioinoculants has been seed coating (Gaur 1990; Khan et al., 2007), under which the target seeds are coated by dipping in the liquid inoculum or coated with the carrier-based fungal inoculants. Through this process the fungi stick to the surface of seeds. However, this process of inoculum application has many disadvantages like the coated culture count may not be sufficient, poor survivability of the inoculated strains, and departure of inocula from rhizosphere.

These problems can be mitigated by the application of an adhesive material like gum Arabic. Another mode of bioinoculant application is soil drenching where the bioinoculant in the form of liquid culture is directly applied to the soil. There are many advantages associated with this method like higher concentration of fungi per unit area, less exposure to chemically treated seeds, less time taken, and finally the inoculants can handle low moisture conditions better than with carrier-based bioinoculants.

15.5 EFFECT OF INOCULATION ON PLANT GROWTH AND INTERACTION WITH OTHER MICROORGANISMS

In this section, we highlight the role of *Penicillium* as PGP fungi, when employed as single or mixed cultures with other microbes, in enhancing the growth and productivity of plants grown in various parts of the world. The role of *Penicillium* as bioinoculant in compost preparation and in the process of composting is long since known (Gaur, 1982; Singh and Nain, 2014). However, the role of *Penicillium* as phosphatic biofertilizer as bionoculant for PGP has recently been investigated.

In an investigation of Aligarh soil several filamentous fungi including *Penicillum* were found to be potential PGP agents (Imran, 2009). Alam et al. (2011) investigated the effect of a PGP fungus, *Penicillium* sp. EU0013, on Fusarium wilt disease. In dual-culture experiments, EU0013 inhibited the growth of Fusarium wilt pathogens by producing an inhibition zone. In trials with sterile potting medium under controlled conditions, EU0013

significantly reduced the severity of Fusarium wilt on tomato (*Solanum lycopersicum* L.) and cabbage (*Brassica oleracea* L. var. *capitata*). In nonsterile soil, benomyl-resistant mutants of EU0013 were selected by exposing the conidial solution of EU0013 to ultraviolet light. The selected mutant EU0013_90S isolate did not show any distinct differences from EU0013 in colony characteristics, growth rate, or antifungal activity against Fusarium wilt pathogens in dual culture. The effect of EU0013_90S on tomato wilt was studied under greenhouse conditions using nonsterile soil. Two-week old tomato seedlings were dipped in four different concentrations of EU0013_90S conidial suspension (1×10^3, 1×10^4, 1×10^5, and 1×10^6 conidia mL^{-1}). Seedlings were then planted in soil inoculated with either *F. oxysporum f.* sp. *lycopersici* race 1 CU1 or race 2 JCM 12575 (1×10^6 bud-cells g^{-1}). It was observed that the greatest disease suppression occurred when seedlings were dipped in the highest concentration of EU0013_90S conidia. This same inoculum concentration of EU0013_90S also resulted in the highest disease reduction in soil infested with JCM 12575. Higher root colonization with EU0013_90S showed a significant reduction in Fusarium wilt disease, suggesting that colonization by *Penicillium* sp. EU0013_90S is important for efficient biocontrol of these diseases.

Patil et al. (2012) conducted a study to determine the capability of P-solubilizing fungi and phosphorus levels on growth, yield, and nutrient content in maize. The field experiment was conducted to check the effect of P-solubilizing fungus *Penicillium bilaji* and *Penicillium* spp. on the availability of applied P-fertilizer in calcareous soil at MARS, University of Agricultural Sciences, Dharwad during *rabi*/*summer* season of 2009−2010. Seed inoculation with phosphorus-solubilizing fungi along with P_2O_5 levels considerably influenced plant height, number of leaves per plant, dry matter production, cob length, grain weight per cob, 1000 grain weight, grain yield, and tissue nutrient content (N, P, K, Zn, and Fe) at teaseling of leaves and harvest of whole plant and P uptake at harvest. Stover yield was not significantly influenced by various treatments. Higher growth and yield of maize were achieved when P-solubilizing fungi treated along with 100% RD P_2O_5 application compared to 0 and 50% RD P_2O_5. It was concluded that single and dual inoculation along with P-fertilizer resulted in 20% to 23% higher maize yield over control. Soil-inhabiting fungal pathogen *Fusarium oxysporum* often causes severe yield losses in many crops.

Radhakrishnan et al. (2014) examined the PGP and stress-mitigation effects of *Penicillium* species RDA01, NICS01, and DFC01 on sesame (*Sesamum indicum* L.) plants. The fungal isolates NICS01 and DFC01 considerably enhanced shoot length, root length, and fresh and dry seedling weight due to the secretion of various concentrations of amino acids (Asp, Thr, Ser, Asn, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Try, and Arg). *Penicillium* sp. NICS01 increased the amount of chlorophylls, proteins, amino acids, and lignans in the sesame plants more so than in controls. Sesame plant growth was stunted by high soil salinity, and application of the three fungal isolates increased plant survival. The RDA01 and NICS01 strains significantly increased shoot length and fresh and dry seedling weights under salt stress conditions. In addition, an in vitro study of *Penicillium* spp. revealed their antagonistic activity toward the pathogenic fungi *Fusarium* spp. *Fusarium* spp. reduce shoot length, and coinoculation with the NICS01 or DFC01 isolates significantly increased shoot length in infected plants. These results suggest that exogenous application of the *Penicillium* sp. NICS01 can act as a biofertilizer and a biocontrol agent to improve plant growth and enhance plant survival against salt stress and *Fusarium* infection.

Ram et al. (2015) investigated whether phosphorus (P) accessibility restricts crop development in the majority of agricultural soils of northwest India. Helpful rhizosphere microbes like phosphate-solubilizing fungi were reported to augment P availability in soil and improve crop yields. Field experiments were performed during 2009−11 to evaluate the effect of seed inoculation with phosphate-solubilizing fungi *Penicillium bilaii* at different rates of fertilizer P on P content in leaves and grain production of irrigated wheat in India. The soil was low in Olsen P at the Bathinda site and medium at the Ludhiana site. In no-P treatment, phosphate-solubilizing fungi significantly increased grain yield by 12.6% over noninoculated control. The effect of phosphate-solubilizing fungi on grain yield was generally more pronounced in soil with low Olsen-P compared to medium Olsen-P level. Inoculation of PSF along with 50% P fertilizer increased wheat yield equivalent to 100% P with no phosphate-solubilizing fungi. Spike density was significantly higher in phosphate-solubilizing fungi + 50% P than all other treatments. There is a need to study the long-term effect of *Penicillium bilaii* on P-fertilizer saving in wheat on soils varying in P availability, pH, and P fixation capacity for different wheat-based cropping systems. In the same year, Babu et al. (2015) reported on the isolation of *Penicillium menonorum* from rhizosphere soil in Korea and its identification based on morphological characteristics and ITS gene sequence. The fungal isolate was named KNU-3 and was found to exhibit PGP activity through IAA and siderophore production, as well as P solubilization. KNU-3 produced 9.7 mg/L IAA and solubilized 408 $mg/^{L}$ of Ca_3PO_4 and inoculation with the isolate significantly ($P < 0.05$) increased the dry biomass of cucumber roots (57%) and shoots (52%). Chlorophyll, starch, protein, and P contents were increased by 16%, 45%, 22%, and 14%, respectively, compared to plants

grown in uninoculated soil. The fungus also increased soil dehydrogenase (30%) and acid phosphatase (19%) activities. These results show that the isolate KNU-3 has potential PGP attributes and thus can be considered as a new fungus to enhance soil fertility and promote plant growth. In this study, PGP by *Penicillium menonorum* KNU-3 was reported for the first time in Korea after its original description.

Bhatt et al. (2016) carried out filed experiments with the objective to investigate the effect of two strains of *Penicillium bilaii* (PB-201 and PB-208) inoculation along with superphosphate application on growth, yield, and P uptake of wheat (cv.PBW-343) and also to examine the inoculation effect on P availability, forms of P, and soil properties in Mollisols of Uttarakhand, India. The results showed that both strains of *P. bilaii* effectively solubilized tri-calcium phosphate in Pikovskaya agar medium, which was much higher than native fungal isolates. Wheat-seed inoculation with *P. bilaii* strains along with superphosphate levels significantly influenced shoot height, shoot dry weight, number of total and effective tillers, yield attributes, yield components, tissue content, and uptake of P. The treatment T7 (*P. bilaii*, strain PB-208 + 50% P) resulted in the highest shoot height (87.9 cm at 90 DAS), shoot dry weight (1.5 and 3.8 g at 60 and 90 DAS, respectively), grain (66.8 q/ha), and straw yield (42.7 q/ha) and P uptake (26.5 kg/ha). The Olsen-P, organic carbon, dehydrogenase activity, and fungal populations also increased in soil inoculated with *P. bilaii* strains combined with superphosphate application compared to the control soil. The combined application of fungal strains with or without phosphate fertilization has give rise to an antagonistic interaction which leads to decline in yield, tissue content and uptake of P and its availability in soil. In conclusion, it is possible to reduce the rate of soluble P-fertilizer added by 50% without reducing yield, if wheat is inoculated with P-solubilizing fungi like *P. bilaii*

15.6 CONCLUSION AND FUTURE PROSPECTS

In today's agricultural scenario the excessive use of chemical fertilizers and other agrochemicals create a serious risk to soil and plant health. Using naturally occurring materials in the form of microbes like PGP microorganisms including free-living fungi can create new prospects for improved plant health together with the preservation of the ecosystem. However, the feasibility of this technology (PGPF) requires the distribution and application of better quality fungal bioinoculants that can satisfy the needs of present farming communities under changing environmental conditions. Further improvement in *Penicillium*-based bioinoculant is needed to explore its performance under different agroclimatic zone and stress conditions. On the other hand, synergistic interaction with other plants and root-associated microbes should be explored for better understanding of its ecological fitness in inoculated plants.

CONFLICT OF INTEREST

None.

References

AbdAlla, M.H., 1994. Phosphatases and the utilization of organic phosphorus by *Rhizobiumleguminosarum* biovar *viceae*. Lett. Appl. Microbiol. 18, 294–296.

Abd-Alla, M.H., El-Enany, A.W.E., Nafady, N.A., Khalaf, D.M., Morsy, F.M., 2014. Synergistic interaction of *Rhizobium leguminosarum* bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiol. Res. 169, 49–58.

Ahuja, A., Ghosh, S.B., D'Souza, S.F., 2007. Isolation of a starch utilizing, phosphate solubilizing fungus on buffered medium and its characterization. Bioresour. Technol. 98, 3408–3411.

Alam, S.S., Sakamoto, K., Inubushi, K., 2011. Biocontrol efficiency of fusarium wilt diseases by a root-colonizing fungus *Penicillium* sp. Soil Sci. Plant Nutr. 57, 204–212.

Alves, M.H., Campos-Takaki, G.M., Porto, A.L.F., Milanez, A.I., 2002. Screening of *mucor* spp. for the production of amylase, lipase, polygalacturonase and protease. Braz. J. Microbiol. 33, 325–330.

Arantes, V., Milagres, A.M.F., 2006. Evaluation of different carbon sources for production of iron-reducing compounds by *Wolfiporia cocos* and *Perenniporia medulla-panis*. Process Biochem. 41, 887–891.

Asea, P.E.A., Kucey, R.M.N., Stewart, J.W.B., 1988. Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Soil Biol. Biochem. 20, 459–464.

Azcon-Aguilar, C., Diaz-Rodriguez, R., Barea, J.M., 1986. Effect of soil microorganisms on spore germination and growth on the vesicular arbuscular mycorrhizal fungus (*Glomus mosseae*). Trans. Br. Mycol. Soc. 86, 337–340.

Baakza, A., Vala, A.K., Dave, B.P., Dube, H.C., 2004. A comparative study of siderophoreproduction by fungi from marine and terrestrial habitats. J. Exp. Mar. Biol. Ecol. 311, 1–9.
Babu, A.G., Kim, S.W., Yadav, D.R., Hyum, U., Adhikari, M., Lee, Y.S., 2015. *Penicillium menonorum*: a novel fungus to promote growth and nutrient management in cucumber plants. Mycobiology 43, 49–56.
Barea, J.M., 2000. Rhizosphere and mycorrhiza of field crops. In: Bala'zs, E., Galante, E., Lynch, J.M., Schepers, J.S., Toutant, J.P., Werner, D., et al.,Biological Resource Management: Connecting Science and Policy. Springer, New York, pp. 110–115.
Barea, J.M., Azco'n, R., Azco'n-Aguilar, C., 2004. Mycorrhizal fungi and plant growth promoting rhizobacteria. In: Varma, A., Abbott, L., Werner, D., Hampp (Eds.), Plant Surface Microbiology. Springer, Germany, pp. 351–371.
Bending, G.D., Read, D.J., 1996. Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil. Biol. Biochem. 28, 1603–1612.
Berbee, M.L., Taylor, J.W., 1999. Fungal phylogeny. In: Oliver, R.P., Schweiser, M. (Eds.), Molecular Fungal Biology. Cambridge University Press, Cambridge, p. 377.
Berbee, M.L., Yoshimura, A., Sugiyama, J., Taylor, J.W., 1995. Is *Penicillium* Monophyletic? An evaluation of phylogeny in the family Trichocomaceae from 18S, 5.8S and ITS ribosomal DNA sequence data. Mycologia. 87, 210–222.
Bhatt, S.C., Devnath, S., Pareek, N., 2016. Assessment of *Penicillium bilaii* inoculation in wheat (*Triticum aestivum* L.) for improving growth, yield and phosphorus availability in Mollisols of India. J. Appl. Nat. Sci. 8, 358–367.
Bhuyan, S.K., Bandyopadhyay, P., Kumar, P., Kumar, P., Mishra, D., Prasad, R., et al., 2015. Interaction of *Piriformospora indica* with *Azotobacter chroococcum*. Sci. Rep. 5, 13911.
Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., 2005. Defining operational taxonomic units using DNA barcode data. Philos. Trans R Soc Lond B Biol. Sci. 360, 1935–1943.
Boswell, G.P., Jacobs, H., Davidson, F.A., Gadd, G.M., Ritz, K., 2003. Growth and function of fungal mycelia in heterogeneous environments. Bull. Math. Biol. 65, 447–477.
Boswell, G.P., Jacobs, H., Ritz, K., Gadd, G.M., Davidson, F.A., 2006. The development of fungal networks in complex environments. Bull. Math. Biol. 69, 605–634.
Bowen, G.D., Rovira, A.D., 1999. The rhizosphere and its management to improve plant growth. Adv. Agronomy 66, 1–102.
Branco de Freitas Maia, C.M., Novotny, E.H., Rittl, T.F., Hayes, B., Hilary, M., 2013. Soil organic matter: chemical and physical characteristics and analytical methods. A review. Curr. Organic Chem. 17, 2985–2990.
Bridge, P., Spooner, B., 2001. Soil fungi: diversity and detection. Plant. Soil. 232, 147–154.
Buckley, N.G., Pugh, G.J., 1971. Auxin production by phylloplane fungi. Nature. 231, 332.
Chai, B., Wu, Y., Liu, P., Liu, B., Gao, M., 2011. Isolation and phosphate-solubilizing ability of a fungus, *Penicillium* sp. from soil of an alum mine. J. Basic. Microbiol. 51, 5–14.
Chen, A.J., Tang, D., Zhou, Y.Q., Sun, B.D., Li, X.J., Wang, L.Z., et al., 2013. Identification of Ochratoxin A producing fungi associated with fresh and dry liquorice. PLoS. ONE. 8, e78285.
Christensen, M., Frisvad, J.C., Tuthill, D.E., 2000. *Penicillium* species diversity in soil and some taxonomic and ecological notes. In: Samson, S.R., Pitt, J.I. (Eds.), Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. Harwood Academic Publishers, Amsterdam, pp. 309–321.
Ciegler, A., Pitt, J.I., 1970. Survey of the genus *Penicillium* for tremorgenic toxin production. Mycopathol. Mycol. Appl. 42, 119–124.
Colpaert, B.J., Laere, A.V., 1996. A comparison of the extracellular enzyme activities of two ectomycorrhizal and a leaf-saprotrophic basidiomycete colonizing beech leaf litter. New Phytol. 133, 133–141.
Courty, P.E., Franc, A., Pierrat, J.C., Garbaye, J., 2008. Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. Appl. Environ. Microbiol. 74, 5792–5801.
Crueger, W., Crueger, A., 1990. Biotechnology: A Textbook of Industrial Microbiology. Sinaeur Associates, Sunderland, MA.
Cunningham, J.E., Kuiack, C., 1992. Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaji*. Appl. Environ. Microbiol. 58, 1451–1458.
De Luca, N.G., Wood, P.M., 2000. Iron uptake by fungi: contrasted mechanisms with internal or external reduction. Adv. Microb. Physiol. 43, 39–74.
De Oliveira Mendes, G., Moreira de Freitas, A.L., Liparini Pereira, O., Ribeiro da Silva, I., Bojkov Vassilev, N., Dutra Costa, M., 2014. Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources. Ann. Microbiol. 1, 239–249.
De Vleesschauwer, D., Xu, J., Höfte, M., 2014. Making sense of hormone-mediated defense networking: from rice to Arabidopsis. Front. Plant Sci. 5, 1–15.
Dube, H.C., Vala, A.K., Vaidya, S.Y., 2000. Chemical nature and ligant binding properties of siderophores produced by certain Aspergilli from marine habitats. Natl. Acad. Sci. Lett. India 23, 98–100.
El-Banna, A.A., Pitt, J.I., Leistner, L., 1987. Production of mycotoxins by *Penicillium* species. Syst. Appl. Microbiol. 10, 42–46.
FAI, 2002. The fertilizer association of India, New Delhi, Fertilizer statistics 2001-02. 1, 39–47.
Fleming, A., 1929. On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. Br. J. Exp. Pathol. 10, 226–236.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63, 433–462.
Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., et al., 2011. Solutions for a cultivated planet. Nature. 478, 337–342.
Fomina, M., Hillier, S., Chamock, J.M., Melville, K., Alexander, I.J., Gadd, G.M., 2005a. Role of oxalic acid over-excretion in toxic metal mineral transformations by *Beauveriacaledonica*. Appl. Environ. Microbiol. 71, 371–381.
Fomina, M., Burford, E.P., Gadd, G.M., 2005b. Toxic metals and fungal communities. In: Dighton, J., White, J.F., Oudemans, P. (Eds.), The Fungal Community: Its Organization Androle in the Ecosystem. CRC Press, Boca Raton, pp. 733–758.
Frisvad, J.C., Samson, R.A., 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud. Mycol. 49, 1–174.

Frisvad, J.C., Smedsgaard, J., Larsen, T.O., Samson, R.A., 2004. Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Stud. Mycol. 49, 201–241.
Fu, S.F., Wei, J.Y., Chen, H.W., Liu, Y.Y., Lu, H.Y., Chou, J.Y., 2015. Indole-3- acetic acid: a widespread physiological code in interactions of fungi with other organisms. Plant Signal. Behav. 10, e1048052.
Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122, 109–119.
Gadd, G.M., 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol. Res. 111, 3–49.
Gadd, G.M., 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology. 156, 609–643.
Gadd, G.M., John, A.R., 2010. Geomicrobiology of eukaryotic microorganisms. Geomicrobiol. J. 27 (6-7), 491–519.
Gadd, G.M., Dyer, P., Watkinson, S., 2007. Fungi in the Environment. Cambridge University Press, Cambridge.
Garbeva, J.A., van Veen, van Elsas, J.D., 2004. Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol. 42, 243–270.
Gaur, A.C., 1982. Role of mesophilic fungi in composting. Agric. Wastes 4, 453–460.
Gaur, A.C., 1990. Phosphate Solubilizing Microorganisms as Biofertilizers. Omega Scientific Publisher, New Delhi.
Geisen, R., Cantor, M.D., Hansen, T.K., Holzapfel, W.H., Jakobsen, M., 2001. Characterization of *Penicillium roqueforti* strains used as cheese starter cultures by RAPD typing. Int. J. Food. Microbiol. 65, 183–191.
Giraud, F., Giraud, T., Aguileta, G., Fournier, E., Samson, R., Cruaud, C., et al., 2010. Microsatellite loci to recognize species for the cheese starter and contaminating strains associated with cheese manufacturing. Int. J. Food. Microbiol. 137, 204–213.
Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41, 109–117.
Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. Am. J. Altern. Agric. 1, 51–57.
Gorbushina, A.A., Krumbein, W.E., Hamann, R., Panina, L., Soucharjevsky, S., Wollenzien, U., 1993. On the role of black fungi in color change and biodeterioration of antique marbles. Geomicrobiol. J. 11, 205–221.
Gorbushina, A.A., Whitehead, K., Domieden, T., Niesse, A., Schulte, A., Hedges, J.I., 2003. Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. Can. J. Botany 81, 131–138.
Gorbushina, A.A., 2007. Life on the rocks. Environ. Microbiol. 9, 1613–1631.
Gruen, H.E., 1959. Auxins and fungi. Annu. Rev. Plant. Physiol. 10, 405–440.
Gupta, N., Sabat, J., Parida, R., Kerkatta, D., 2007. Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. Acta Botanica Croatica 66, 197–204.
Halder, A.K., Mishra, A.K., Chakarbarthy, P.K., 1991. Solubilization of inorganic phosphate by *Bradyrhizobium*. Indian. J. Exp. Biol. 29, 28–31.
Hasan, H.A., 2002. Gibberellin and auxin-indole production by plant root-fungi and their biosynthesis under salinity-calcium interaction. Acta. Microbiol. Immunol. Hung. 49 (1), 105–118.
Hider, 1984. Siderophore mediated absorption of iron. Siderophores from microorganisms and plants. Struct. Bond. 58, 25–87.
Houbraken, J., Samson, R.A., 2011. Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. Stud. Mycol. 70, 1–51.
Houbraken, J., Frisvad, J.C., Seifert, K.A., Overy, D.P., Tuthill, D.M., Valdez, J.G., et al., 2012. New penicillin-producing *Penicillium* species and an overview of section Chrysogena. Persoonia 29, 78–100.
Imran, M., 2009. Interaction of heavy metals with indigenous isolates of free living rhizospheric fungi and their plant growth promoting potentials. Unpublished doctoral thesis. Aligarh Muslim University, Aligarh.
Khan, M.S., Zaidi, A., Wani, P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture - a review. Agronomy Sustain. Dev. 27, 29–43.
Khan, M.S., Zaidi, A., Ahemad, M., Oves, M., Wani, P.A., 2010. Plant growth promotion by phosphate solubilizing fungi-current perspective. Arch. Agronomy Soil Sci. 56, 73–98.
Kirk, T.K., Farrell, R.L., 1987. Enzymatic 'combustion': the microbial degradation of lignin. Annu. Rev. Microbiol. 41, 465–505.
Koljalg, U., Nilsson, R.H., Abarenkov, K., Tedersso, L., Taylor, A.F.S., Bahram, M., et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Korat, K., Dave, B.P., Dube, H.C., 2001. Detection and chemical characterization of siderophore produced by certain fungi. Indian J. Microbiol. 41, 87–91.
Kornerup, A., Wanscher, J.H., 1967. Methuen Handbook of Color. Methuen & Co Ltd, Copenhagen.
Lagos, M.L., Maruyama, F., Nannipieri, P., Mora, M.L., Ogram, A., Jorquera, M.A., 2015. Current overview on the study of bacteria in the rhizosphere by modern molecular techniques: a mini-review. J. Soil Sci. Plant Nutr. 15, 504–523.
Lehninger, A.L., 1988. Principios de Bioquimica. Sarvier, Sao Paulo.
Li, Y., Cui, F., Liu, Z., Xu, Yingying, Zhao, H., 2007. Improvement of xylanase production by *Penicillium oxalicum* ZH-30 using response surface methodology. Enzyme. Microb. Technol. 40, 1381–1388.
Lima, V.A., Aquarone, E., Barzani, V., 1986. Biotechnologia-technologia das fermentacois. Edgard blucher Ltd, Sao Paulo.
Link, H.F., 1809. Observationes in Ordines plantarum naturales. Dissertatio 1ma. Magazin der Gesellschaft Naturforschenden Freunde Berlin 3, 3–42.
LoBuglio, K.F., Pitt, J.I., Taylor, J.W., 1993. Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of a sexual Talaromyces state among asexual *Penicillium* species in subgenus *Biverticillium*. Mycologica 85, 592–604.
Lopez-Díaz, T.M., Santos, J.A., García-Lopez, M.L., Otero, A., 2001. Surface mycoflora of a Spanish fermented meat sausage and toxigenicity of *Penicillium* isolates. Int. J. Food. Microbiol. 68, 69–74.
Ludemann, V., Greco, M., Rodríguez, M.P., Juan Carlos Basílico, J.C., Pardo, A.G., 2010. Conidial production by *Penicillium nalgiovense* for use as starter cultures in dry fermented sausages by solid state fermentation. LWT Food Sci. Technol. 43, 315–318.
McNeill, J., Barrie, F.F., Buck, W.R., Demoulin, V., Greuter, W., Hawsworth, H.L. (Eds.), 2012. International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code). Koeltz Scientific Books, Königstein [Regnum vegetabile no. 154.].
Nahas, E., 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. World J. Microbiol. Biotechnol. 12, 567–572.

Neilands, J.B., 1984. Methodology of siderophores. Siderophores from microorganisms and plants. Struct. Bond. 58, 1–24.
Neilands, J.B., 1993. Perspectives in biochemistry and biophysics. Siderophores. Arch. Biochem. Biophys. 302, 1–3.
Neilands, J.B., 1995. Siderophores: structure and function of microbial iron transport compounds. J. Biol. Chem. 270, 26723–26726.
Nielsen, R.I., Oxenbøll, K., 1998. Enzymes from fungi: their technology and uses. Mycologist 12, 69–71.
O'Brien, H., Parrent, J., Jackson, J., Moncalvo, J., Vilgalys, R., 2005. Fungal community analysis by largescale sequencing of environmental samples. Appl. Environ. Microbiol. 71, 5544–5550.
Okuda, T., 1994. Variation in colony characteristics of *Penicillium* strains resulting from minor variations in culture conditions. Mycologia. 86, 259–262.
Okuda, T., Klich, M.A., Seifert, K.A., Ando, K., 2000. Media and incubation effect on morphological characteristics of *Penicillium* and *Aspergillus*. In: Samson, R.A., Pitt, J.I. (Eds.), Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. Harwood Academic Publishers, Amsterdam, pp. 83–99.
Omar, S.A., 1998. The role of rock-phosphate-solubilizing fungi and vesicular arbusular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol. 14, 211–218.
Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., Mohan, R., 2000. Advances in microbial amylases. Biotechnol. Appl. Biochem. 31, 135–152.
Paterson, R.R., Bridge, P.D., Crosswaite, M.J., HAawksworth, D.L., 1989. A reappraisal of the terverticillate Penicillia using biochemical, physiological and morphological features iii. An evaluation of pectinase and amylase isoenzymes for species characterization. J. Gen. Microbiol. 135, 2979–2991.
Patil, P.M., Kuligod, V.B., Hebsur, N.S., Patil, C.R., Kulkarni, G.N., 2012. Effect of phosphate solubilizing fungi and phosphorus levels on growth, yield and nutrient content in maize (*Zea mays*). Karnataka J. Agric. Sci. 25, 58–62.
Peleg, Z., Reguera, M., Tumimbang, E., Walia, H., Blumwald, E., 2011. Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. Plant. Biotechnol. J. 9, 747–758.
Perez-Miranda, S., Cabirol, N., George-Tellez, R., Zamudio-Rivera, L.S., Fernandez, F.J., 2007. 0-CAS, a fast and universal method for siderophore detection. J. Microbiol. Methods. 70 (1), 127–131.
Peterson, S.W., Vega, F., Posada, F., Nagai, C., 2005. *Penicillium coffeae*, a new endophytic species isolated from a coffee plant and its phylogenetic relationship *to P. fellutanum, P. thiersii* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. Mycologia. 97, 659–666.
Pitt, J.I., 1973. An appraisal of identification methods for *Penicillium* species Novel taxonomic criteria based on temperature and water relations. Mycologia. 65, 1135–1157.
Pitt, J.I., 1979. The Genus *Penicillium* and Its Teleomorphic States Eupenicillium and Talaromyces. Academic Press Inc, London.
Pitt, J.I., Samson, R.A., 1993. Names in current use in the family Trichocomaceae. In: Greuter, W. (Ed.), Names in Current Use in the Family Trichocomaceae, Cladoniaceae, Pinaceae, *and* Lemnaceae. Koeltz Scientific Books, Konigstein, pp. 13–57.
Pitt, J.I., Samson, R.A., Frisvad, J.C., 2000. List of accepted species and their synonyms in the family Trichocomaceae. In: Samson, R.A., Pitt, J.I. (Eds.), Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. Harwood Academic Publishers, Amsterdam, pp. 9–79.
Pozo, M.J., López-Ráez, J.A., Azcón-Aguilar, C., García-Garrido, J.M., 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytol. 205, 1431–1436.
Radhakrishnan, R., Kang, S.-M., Baek, I.-Y., Lee, I.-J., 2014. Characterization of plant growth-promoting traits of *Penicillium* species against the effects of high soil salinity and root disease. J. Plant Interact. 9, 754–762.
Ram, H., Malik, S.S., Dhaliwal, S.S., Kumar, B., Singh, Y., 2015. Growth and productivity of wheat affected by phosphorus-solubilizing fungi and phosphorus levels. Plant Soil Environ. 61, 122–126.
Raman, K.V., 2005. Sustaining soil fertility. The Hindu Survey of Indian Agriculture 165–167.
Raper, K.B., Thom, C., 1949. A Manual of the Penicillia. The Williams & Wilkins Company, Baltimore.
Ratledge, C., Dover, L.G., 2000. Iron metabolism in pathogenic bacteria. Annu. Rev. Microbiol. 54, 881–941.
Reddy, M.S., Kumar, S.K., Babita, M.S.R., 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. Bioresour. Technol. 84, 187–189.
Ridgeway, R., 1912. Color standards and color nomenclature. Washington DC.
Rincon-Florez, V.A., Carvalhais, L.C., Schenk, P.M., 2013. Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5, 581–612.
Robson, G., 1999. Hyphal cell biology. In: Schweizer, M. (Ed.), Molecular Fungal Biology. Cambridge University Press, Cambridge.
Saha, M., Sarkar, S., Sarkar, B., Sharma, B.K., Bhattacharjee, S., Tribedi, P., 2016. Microbial siderophores and their potential applications: a review. Environ. Sci. Pollut. Res. 23, 3984–3999.
Saldajeno, M.G.B., Hyakumachi, M., 2011. The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus *Glomus mosseae* stimulate plant growth and reduce severity of anthracnose and damping-off diseases in cucumber (*Cucumis sativus*) seedlings. Ann. Appl. Biol. 159, 28–40.
Samson R.A., Houbraken J., Thrane U., Frisvad J.C., Andersen B., Food and Indoor Fungi, 2010, CBS KNAW Biodiversity Center; Utrecht.
Samson, R.A., Pitt, J.I., 1985. General recommendations. In: Samson, R.A., Pitt, J.I. (Eds.), Advances in *Penicillium* and *Aspergillus* Systematics. Plenum Press, London, pp. 455–460.
Samson, R.A., Yilmaz, N., Houbraken, J., Spierenburg, H., Seifert, K.A., Peterson, S.W., et al., 2011. Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in *Penicillium* subgenus *Biverticillium*. Stud. Mycol. 70, 159–183.
Santamaria, M., Fosso, B., Consiglio, A., De Caro, G., Grillo, G., Licciulli, F., et al., 2012. Reference databases for taxonomic assignment in metagenomics. Brief. Bioinform. 13, 682–695.
Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, W., Spouge, J.L., Levesque, C.A., et al., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc. Natl. Acad. Sci. USA 109, 6241–6246.
Schoch, C.L., Robbertse, B., Robert, V., Vu, D., Cardinali, G., Irinyi, L., et al., 2014. Finding needles in haystacks: linking scientific names, reference specimens and molecular data for fungi. Database (Oxford) 2014, bau061. Available from: http://dx.doi.org/10.1093/database/bau061.

Seifert, K.A., Samson, R.A., deWaard, J.R., Houbraken, J., Lévesque, C.A., Moncalvo, J.M., et al., 2007. Prospects for fungus identification using CO1 DNA barcodes, with *Penicillium* as a test case. Proc. Natl. Acad. Sci. USA 104, 3901–3906.
Serra, R., Peterson, S.W., CTCOR, Venâncio, A., 2008. Multilocus sequence identification of *Penicillium* species in cork bark during plank preparation for the manufacture of stoppers. Res. Microbiol. 159, 178–186.
Singh, D.P., Singh, H.B., 2008. Microbial wealth regulates crop quality and soil health. LEISA India 10, 25–26.
Singh, S., Nain, L., 2014. Microorganisms in the conversion of agricultural wastes to compost. Proc. Indian Natl Sci. Acad. 80 (2), 473–481.
Skouboe, P., Frisvad, J.C., Taylor, J.W., Lauritsen, D., Boysen, M., Rossen, L., 1999. Phylogenetic analysis of nucleotide sequences from the ITS region of terverticillate *Penicillium* species. Mycol. Res. 103, 873–881.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Academic Press, London.
Spence, C.A., Lakshmanan, V., Donofrio, N., Bais, H.P., 2015. Crucial roles of abscisic acid biogenesis in virulence of rice blast fungus *Magnaporthe oryzae*. Front. Plant Sci. 6, 1–13.
Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbet, D.S., et al., 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 31, 21–32.
Terrasan, C.R.F., Temer, B., Duarte, M.C.T., Carmona, E.C., 2010. Production of xylanolytic enzymes by *Penicillium janczewskii*. Bioresour. Technol. 101, 4139–4143.
Thom, C., 1930. *The* Penicillia. The Williams & Wilkins Company, Baltimore.
Thom, C., 1954. The evolution of species concepts in *Aspergillus* and *Penicillium*. Ann. N. Y. Acad. Sci. 60, 24–34.
Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature. 418, 671–677.
Torsvik, V., Ovreas, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. Curr. Opin. Microbiol. 5, 240–245.
Tudzynski, B., Sharon, A., 2002. Biosynthesis, biological role and application of fungal phytohormones. In: Osiewacz, H. (Ed.), The Mycota. A Comprehensive Treatise on Flingi as Experimental Systems for Basic and Applied Research- Industrial Applications. Springer, Germany, pp. 183–211.
Vala, A.K., Dave, B.P., Dube, H.C., 2006. Chemical characterization and quantification of siderophores produced by marine and terrestrial *Aspergilli*. Can. J. Microbiol. 52, 603–607.
Van Elsas, J.D., Trevors, J.T., 1997. Modem Soil Microbiology. Marcel Dekker, New York.
Vassileva, M., Azcon, R., Barea, J.M., Vassilev, N., 1998. Application of an encapsulated filamentous fungus in solubilization of inorganic phosphate. J. Biotechnol. 63, 67–72.
Venkateswarlu, B., Rao, A.V., Raina, P., Ahmad, N., 1984. Evaluation of phosphorus solubilization by microorganisms isolated from arid soil. J. Indian Soc. Soil Sci. 32, 273–277.
Visagie, C.M., Houbraken, J., Rodriques, C., Pereira, C.S., Dijksterhuis, J., Seifert, K.A., et al., 2013. Five new *Penicillium* species in section Sclerotiora: a tribute to the Dutch Royal family. Persoonia 31, 42–62.
Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S.B., Klaassen, C.H.W., Perrone, G., et al., 2014. Identification and nomenclature of the genus *Penicillium*. Stud. Mycol. 78, 343–371.
Vyas, P., Rahi, P., Chauhan, A., Gulati, A., 2007. Phosphate solubilization potential and stress tolerance of *Eupenicillium parvum* from tea soil. Mycol. Res. 111, 931–938.
Wakelin, S.A., Warren, R.A., Harvey, P.R., Ryder, M.H., 2004. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol. Fertil. Soils 40, 36–43.
Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D., Tilman, D., 2006. Resource availability controls fungal diversity across a plant diversity gradient. Ecol. Lett. 9, 1127–1135.
Wang, B., Wang, L., 2013. *Penicillium kongii*, a new terverticillate species isolated from plant leaves in China. Mycologia. 105, 1547–1554.
Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67, 321–358.
Whitelaw, M.A., 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv. Agronomy 69, 99–151.
Whitelaw, M.A., Harden, T.J., Helyar, K.R., 1999. Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. Soil Biol. Biochem. 32, 655–665.
Wiebe, M.G., 2003. Stable production of recombinant proteins in filamentous fungi – problems and improvements. Mycologist 17, 140–144.
Wilkins, M.B., Cane, A.R., McCorquodole, I., 1972. Auxin transport in roots, IX. Movement export, respiration and loss of radioactivity from lAA by zea root segments. Planta 106, 291–310.
Winkelmann, G., 2002. Microbial siderophores-mediated transport. Biochem. Soc. Trans. 30, 691–695.
Yin, Z., Shi, F., Jiang, H., Roberts, D.P., Chen, S., Fan, B., 2015. Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareus soil. Can. J. Microbiol. 61, 913–923.
Zheng-ji, H., Zheng, Y., Chen, C.R., 2008. Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture dependent and culture-independent approaches. J. Soils Sediments 8, 349–358.

Further Reading

Blaxter, M., 2003. Counting angels with DNA. Nature. 421, 1–3.
Thom, C., 1945. Mycology present penicillin. Mycologia. 37, 460–475.
Wahid, O.A., Mehana, T.A., 2000. Impact of phosphate solubilizing fungi on the yield and phosphorus uptake by wheat and faba bean plants. Microbiol. Res. 155, 221–227.
Winkelmaim, G., 1992. Structures and functions of fungal siderophores containing hydroxamate and complexone type iron binding ligands. Mycol. Res. 96, 529–534.