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REVIEW

Piriformospora indica: A Novel Plant Growth-Promoting Mycorrhizal Fungus

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Abstract *Piriformospora indica,* a member of the newly created order Sebacinales, is extremely versatile in its mycorrhizal associations and its ability to promote plant growth. *P. indica* is widely distributed as a symptomless root endophyte, and it colonizes members of bryophytes, pteridophytes, gymnosperms and angiosperms. *P. indica* and allied members of Sebacinales have been reported to occur in four continents. The existing literature suggests that the multitude of mycorrhizal interactions in Sebacinales may have arisen from an ancestral endophytic habitat by specialization. Considering their proven beneficial influence on plant growth and their ubiquity, endophytic *P. indica* may have been a previously unrecognized universal hidden force in plant ecosystems. Root colonization by *P. indica* results in an increase in plant growth, early flowering, higher seed yield, alteration in the secondary metabolites, and adaptation to abiotic and biotic stresses. The colonization of roots begins with a biotrophic growth phase, in which living cells are colonized, and continues with a cell death-dependent phase, in which root cells are actively killed by the fungus. The complexity of sebacinalean symbiosis is further enhanced by the presence of endocellular bacteria which may represent significant determinants for a successful outcome of the symbioses. *P. indica* is shown to have enormous bioprotective potential against plant pathogens and insect pests of agricultural and horticultural crops. Recently, decoding of *P. indica*'s genome has revealed its potential for application as a plant growth-promoting mycorrhizal fungus for realizing the targeted improvement in the production of crop plants.

Keywords *Piriformospora indica* · Plant growth-promoting mycorrhizal fungus · PGPF

Introduction

Piriformospora indica was obtained from the rhizosphere soils of the woody shrubs *Prosopis juliflora* (Swartz) DC. and *Zizyphus nummularia* (Burm. fil.) Wt. & Arn. in the sandy desert soils of Rajasthan, India [48, 52]. The fungus is easily cultivable, lacks host specificity and colonizes roots of many different plants, mostly in an endophytic fashion [49]. *P. indica* is a wide-host root-colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stresses. It interacts with a wide range of hosts, including bryophytes, pteridophytes, gymnosperms and a

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large number of mono- and dicot plants [12, 27–29, 31, 37, 49]. The fungus grows inter- and intracellularly, forms pear-shaped, auto fluorescent chlamydospores within the cortex of the colonized roots and in the rhizosphere zone, but it does not invade the endodermis and the aerial parts of the plants. The fungus promotes nutrient uptake, allows plants to survive under water, temperature and salt stresses, and confers systemic resistance to toxins, heavy metal ions, insects and pathogenic organisms [10]. Further, it is shown to stimulate excessive production of biomass, early flowering, seed production and a potential microorganism imparting biological hardening to tissue culture-raised plants [11, 52, 56]. This article highlights the important biological and molecular features of the fungus and the potential biotechnological applications as a plant growth-promoting mycorrhizal fungus (PGPF).

Taxonomy

Taxonomic position of this fungus is determined by molecular methods based on 18S rRNA sequences and by electron microscopy, which suggest that this fungus is related to the Hymenomycetes of the Basidiomycota. Electron microscopy revealed the presence of typical dolipores with continuous non-perforated parenthesomes, which indicated that *P. indica* belongs to the Hymenomycetes (Basidiomycota). Comparison with sequences from the Genbank data base indicated that *P. indica* is closely related to the *Rhizoctonia* group [49].

Molecular phylogenetic analyses have revealed that *P. indica* is a member of the basidiomycetous order Sebacinales (Basidiomycota: Agaricomycetes) [18, 33, 54]. Until the advent of molecular methods for fungal identification, only a few morphospecies were known from this fungal group. Since then, more and more sebacinoid fungi have been detected as mycobionts of plant roots in molecular ecological studies, and it has become apparent that Sebacinales harbours an enormous biodiversity [55].

Although the exact phylogenetic position of the Sebacinales within the Agaricomycetes is still unclear, it has been shown that they can be divided into two major clades, which have been informally designated as groups A and B (Fig. 1) ([33]; see also [55]). The anamorphic *P. indica*, belongs to group B. Interestingly, *P. indica* is phylogenetically closely related to strain DAR 29830 [55], a multinucleate *Rhizoctonia*. Basiewicz et al. [3] reported significant differences in the physiological and molecular parameters inferred from morphologically very similar strains of *Piriformospora*. As a first taxonomic consequence, they have described *Piriformospora williamsii* as a new member of the so far monotypic genus *Piriformospora* and show that this genus contains still undescribed species that were recently discovered as endophytes of field-collected specimens of *Anthyllis*, *Medicago*, and *Lolium* in Germany.

The complete 24.97 Mb genome of *P. indica* has been sequenced and confirmed by blast search with highly conserved core genes present in higher eukaryotes [57, 58]. The sequence analysis has shown that *P. indica* possesses biotroph-associated genomic adaptations, such as lack of genes involved in nitrogen metabolism and a limited potential for damage and destruction shared by symbiotic fungi and obligate biotrophic pathogens. On the other hand, *P. indica* also shares genomic traits with saprotrophic and hemibiotrophic phytopathogenic fungi, such as the presence of an expanded enzyme arsenal which is weakly expressed during the initial biotrophic phase. These and cytological evidences suggest that *P. indica* represents a missing link between decomposer fungi and obligate biotrophic mutualists [58].

Morphological Features

The young mycelia of *P. indica* are white and almost hyaline, but inconspicuous zonations are observed in older cultures. The mycelia are mostly flat and submerged into the substratum. The hyphae are thin walled and of different diameters ranging from 0.7 to 3.5 µm. The mycelia are often intertwined and overlap each other. In older cultures and on the root surface, hyphae are often irregularly inflated, showing a nodose to coralloid shape, and granulated dense bodies are formed. Hyphae sometimes show anastomosis and are irregularly septated. For this reason, many cells contain more than one nucleus. Chlamydospores are formed from thin-walled vesicles at the tips of the hyphae. The chlamydospores appear singly or in clusters and are distinctive because of their pear-shaped structure. Very young spores have thin, hyaline walls. At maturity, the walls are up to 1.5 µm thick, which appear as two layered: smooth and pale yellow. The cytoplasm of the chlamydospores is densely packed with granular materials and usually contains 8–25 nuclei (Fig. 2). Neither clamp connections nor sexual structures are found.

Culture Characteristics

Piriformospora indica grows best on modified Hill–Käfer synthetic medium [19, 30]. Under the optimized cultural conditions (inoculum size: 5 %; agitation speed: 200 rpm; working volume: 30 %; initial pH: 6.5; temperature: 30 °C), maximum dry cell weight is obtained after 5 days; in 500-ml Erlenmeyer flask, the sporulation starts after 6 days of growth; and maximum spore yield is obtained after 8 days. However, when *P. indica* was grown in a 14-l bioreactor (Chemap AG, Switzerland) using Hill–Käfer medium maximum, dry cell weight was obtained after 42 h of growth, the fungus-initiated sporulation after 48 h, and a

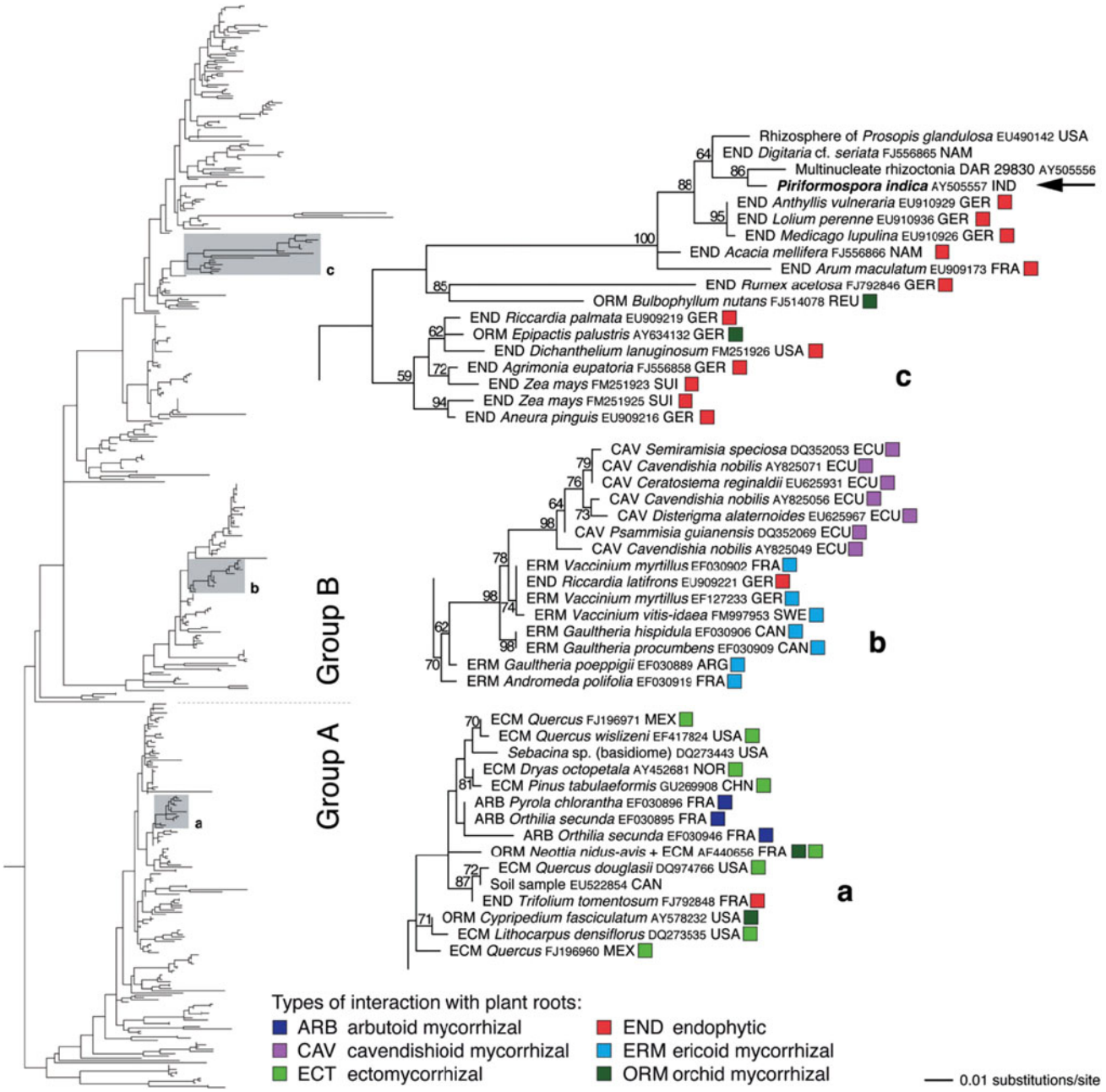

Fig. 1 An overview of the phylogenetic relationships in the Sebacinales, based on a representative sampling of partial nuclear-encoded ribosomal large subunit sequences. Most of the available sequences are from mycobionts detected in plant roots in the field. The alignment was calculated using MAFFT [22]; a phylogenetic hypothesis was constructed using maximum likelihood analysis as implemented in RAxML [42]. Detailed subtrees illustrate the phylogenetic relationships of the three clades *highlighted* in the full tree (a from Sebacinales group A; b, c from group B); subtree sequences are identified by their GenBank accession numbers. The placement of *P. indica* in clade c is indicated by the *arrow*. *Numbers* on the branches are bootstrap support values obtained from 1,000 replicates (only values $\geq$50 % are shown); branch lengths are scaled in terms of the number of expected substitutions per nucleotide. *Coloured boxes* indicate the type of symbiosis. Country codes used: *ARG* Argentina; *CAN* Canada; *CHN* China; *ECU* Ecuador; *FRA* France; *GER* Germany; *GBR* Great Britain; *IND* India; *ITA* Italy; *MEX* Mexico; *NAM* Namibia; *NOR* Norway; *REU* Reunion; *SUI* Switzerland; *USA* United States of America. For more details of Sebacinales phylogeny, see [33]. (Color figure online)

spore yield of 9.25×10^7 spores/ml was achieved after 60 h of growth. The early sporulation in this case may be due to rapid consumption of glucose. Owing to more efficient mixing and homogenized fungal suspension, the growth of fungus was faster in the bioreactor and resulted in early depletion of the carbon source and thereby in the early sporulation compared with the batch culture in shake flasks.

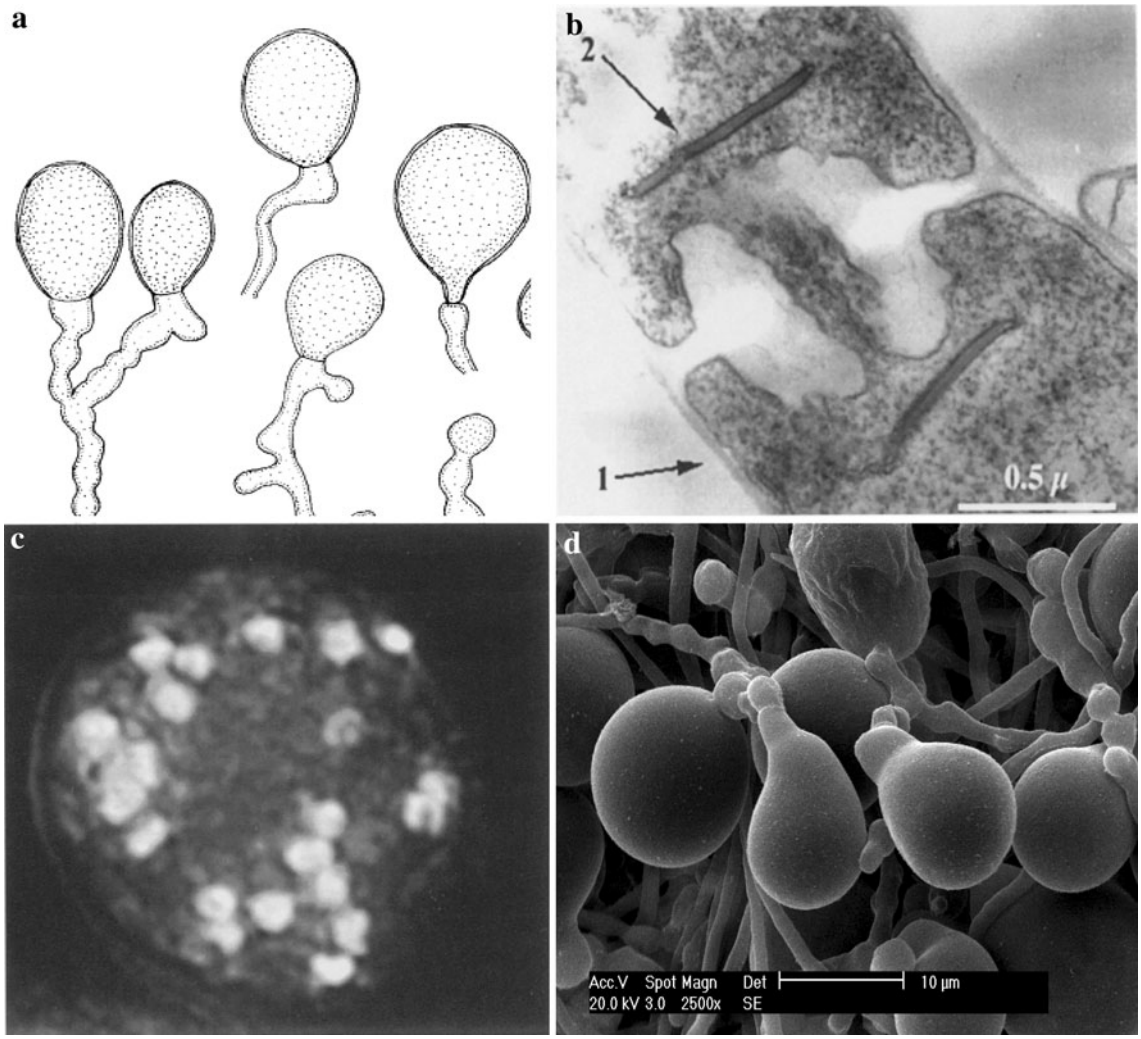

Fig. 2 **a** Chlamydospores of *P. indica*. **b** Ultrathin sections of *P. indica* mycelium showing dolipore, parenthesomes (*arrow 2*) and cell wall (*arrow 1*). **c** Nuclei in a chlamydospore. Chlamydospores were stained with DAPI and observed by epifluorescense microscopy. Different optical plains were assembled in one picture using the Improvision software package (IMPROVISION, Coventry, UK). **d** Scanning electron micrograph of chlamydospores of *P. indica*

The pattern of pH profile was quite similar in all those experiments where complex nitrogen sources were present in the growth medium. The uptake of glucose resulted in acidification of fermentation broth which might be due to generation of acidic metabolites. The growth of the fungus remained unaffected as long as the pH during the log phase was not reduced below 4.5 [4].

Formulation

Effective formulation of *P. indica* for use as bioinoculant is prepared in carrier powder (Magnesium sulphite) or vermiculite with 20 % moisture sterilized by autoclaving at 121 °C temperature and 15 lb/inch^2 pressure for 20 min for three consecutive days. The average particles size is maintained as 50–80 and 40–70 μm for talcum and vermiculite, respectively. The live propagules value is maintained as 10^9 cfu [36].

Interaction with Plant Growth-Promoting Rhizobacteria and Other Rhizobacteria

Exploiting the potential of fluorescent *Pseudomonads, Serratia* spp., *Bacillus* spp. and *Burkholderia* spp. to act as crop protectants (biological control agent) has been the focus of many research groups. Their biocontrol capabilities result largely from their ability to produce a battery of antifungal metabolites which also can affect beneficial fungal-root symbioses [34]. On the other hand, *Azospirillum* and *Herbaspirillum* spp., for example, which are known as diazotrophic, plant growth-promoting agents or green biofertilizer, are also gaining much importance to

improve nutrition, growth and yield of crop and energy plants [13]. Research on the cellular and metabolome basis of the interaction of these plant-beneficial bacteria with *P. indica* should contribute to the understanding of the beneficial or deleterious associations between bacteria and fungi in general. The intense interaction between *P. indica* and economically important rhizobacteria is known. While some rhizobacteria could promote growth and root colonization of the fungus or behave neutral in the interaction with *P. indica*, others severely inhibit its development.

Confrontation Assays on Plates

Stimulatory and inhibitory influences as a result of co-cultivation of *P. indica* with diverse rhizobacteria have been studied in confrontation assays on nutrient agar plates using a collection of strains and mutants. While *Pseudomonas putida* IsoF promoted the growth of the fungus, most of the *Pseudomonas* strains, like *Ps. fluorescens* WS5 and SS101, and the nitrogen-fixing *Burkholderia cepacia* LA3, *Gluconacetobacter* sp. Comb19, and *Streptomyces lividans* SL8 inhibited the growth of *P. indica* (Table 1). The commercially available plant growth-enhancing strain *Bacillus amyloliquefaciens* FZB42 (Rhizo Plus^{R}, ABITEP, Berlin, Germany) has been investigated to determine the nature of the inhibitory compound. In this bacterium, several lipopeptide and antibiotics are known, and knock-out mutants are available [7, 23]. While the wild type strain FZB42 severely inhibits the growth of *P. indica*, the mutants CH3, AK1 and AK3, lacking lipopeptide, polyketide synthesis or bacillomycin D production, respectively, had no inhibitory effect. In contrast, the mutant AK2, deficient only in fengyin production, was still inhibitory. This pointed to the fact, that bacillomycin is the most effective inhibitory metabolite in the interaction of FZB42 with *P. indica*. The biocontrol strain *Ps. fluorescens* SS101 [34] inhibited growth of *P. indica*, while its lipopeptide biosurfactant massetolide A-deficient mutant 10.24 had clearly no inhibitory effect on hyphal growth of *P. indica.* Accordingly, the isolated cyclic lipopeptide massetolide A inhibited the growth of *P. indica* down to 1–10 μg concentrations in the confrontation assay.

Interaction of Rhizobacteria with *P. indica* in Axenic Barley Seedlings

When *P. indica* is applied to barley roots in an axenic system, root development is seen enhanced already in the seedling stage (Fig. 3a). Selected rhizobacteria that showed stimulation or inhibition of the growth of *P. indica* in the confrontation plate assay influenced also the root growth stimulatory effect of *P. indica* in the gnotobiotic barley

Table 1 Influence of rhizobacteria on growth of *P. indica*

Strains	Source	Impact
Herbaspirillum frisingense GSF30^{T}	Muenchen	Neutral
H. lusitanum P6-12^{T}	Muenchen	Neutral
H. seropedicae Z67^{T}	Muenchen	Neutral
H. rubrisubalbicans LMG 2286^{T}	Muenchen	Neutral
Azospirillum brasilense Sp7^{T}	Muenchen	Neutral
A. brasilensis Sp245	Muenchen	Neutral
Bacillus coagulans NCC235	New Delhi	Neutral
Bacillus subtilis NCC09	New Delhi	Neutral
Pseudomonas putida IsoF	Muenchen	Stimulatory
Serratia liquefaciens MG1	Muenchen	Inhibitory
Burkholderia cepacia LA3	Muenchen/Dharbanga	Inhibitory
Ps. fluorescens WS5	Bangalore	Inhibitory
Gluconacetobacter sp. Comb19	Coimbatore	Inhibitory
Streptomyces lividans SL8	Jena	Inhibitory
Streptomyces coelicor A 3(2)	Jena	Inhibitory
Bacillus amyloliquefaciens FZB42	Berlin	Inhibitory
Bacillus amyloliquefaciens FZB42, mutant CH3[a]	Berlin	No inhibition
Bacillus amyloliquefaciens FZB42, mutant AK1[b]	Berlin	No inhibition
Bacillus amyloliquefaciens FZB42, mutant AK2[c]	Berlin	Slight inhibition
Bacillus amyloliquefaciens FZB42, mutant AK3[d]	Berlin	No inhibition
Pseudomonas fluorescens SS101	Wageningen/Netherlands	Inhibitory
Pseudomonas fluorescens SS101, mutant 10.24[e]	Wageningen/Netherlands	No inhibition

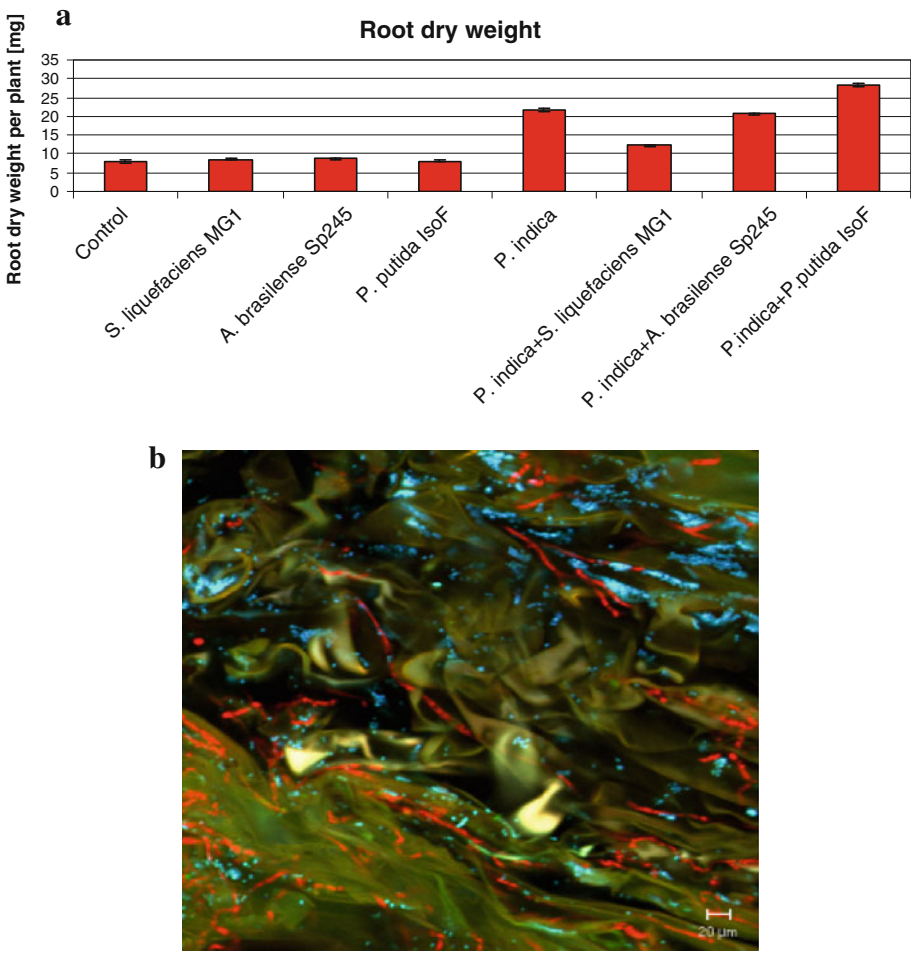

Fig. 3 **a** Root dry weight of axenically grown barley seedlings under the influence of *P. indica* and rhizobacteria *A. brasilense* Sp245, *Serratia liquefaciens* MG1 and *Ps. putida* IsoF (control: not inoculated). **b** Colonization of barley roots in an axenic system by *P. indica* and *Ps. putida* IsoF, demonstrated by FISH-analysis and confocal laser scanning microscopy (LSM510, Zeiss Jena, Germany). The fluorescence labelled oligonucleotide probes Eub339 I, II, III-Cy5 (*blue* in rgb-image) for bacteria (*Ps. putida* IsoF) and EuK-Cy3 (*red* in the rgb-image) for *P. indica* were applied [50]. (Color figure online)

system. While *Azospirillum brasilense* Sp245 had no effect, *Serratia liquefaciens* MG1 clearly inhibited, and *Pseudomonas putida* IsoF enhanced, the root growth stimulation of *P. indica*. FISH-analysis using probes specific for the bacteria and fungi and confocal laser scanning microscopy demonstrated close physical interactions between the *Pseudomonas putida* IsoF and *P. indica* [29] (Fig. 3b), while in the case of inhibitory interactions, close contact was rare (not shown).

Structural Changes of *P. indica* Induced by Inhibitory *Ps. fluorescens* WS5

In vitro inhibition assays on solid agar medium revealed that *Ps. fluorescens* WS5 and *Burkholderia cepacia* LA3 [20] inhibit the growth of *P. indica*. In the presence of *P. fluorescens* WS5, no observable mycelial mat of *P. indica* is produced, the hyphae remain thin, and lysis is observed in some of the hyphae. In addition, a moderate acidification of the medium (from pH 6.5 down to pH 5.5–6.0) is observed in *P. indica* control cultures, while in co-culture with *P. fluorescens* WS5, the pH is reduced to pH < 4.0.

Scanning electron microscopy of *P. indica* showed healthy hyphae with a smooth chitinous cell wall. The fungal hyphae were damaged when the bacterial cells were in direct physical contact with the fungus [50]. Certain dotted substances were released. Transmission electron micrography further unravelled the morphology of the dotted substance released during *Ps. fluorescens* WS5 interaction. At 28000× magnification, the *Pseudomonas*-treated hyphae showed the button-like structure with single central dot. This unique structure is approximately of 20–25 nm in diameter. The nature of these structures remains to be established.

The unit volume of the treated fungus culture was reduced from 20.2 to 11.9 μm^3, where unit length of hyphae was considered 5 µm, and volume is calculated using formula $\pi r^2 h$ (where $\pi = 3.14$, $r =$ radius of the hypha, and $h =$ unit length of the hypha). In several micrographs, it was observed that the number of mitochondria varied from 2 to 7 (considering longitudinal as well as transverse sections) per unit volume hyphae without the influence of *Ps. fluorescens* WS5. In the bacteria-treated hyphae, the number of mitochondria was considerably reduced to 0–3 per 5 μm^3 hyphal length [9].

Metabolome Analysis of *P. indica*

The inhibitory influence of *B. cepacia* LA3 was more intense (0.9-cm growth diameter) as compared to *Ps. fluorescens* WS5 (1.3-cm growth diameter). In the control cultures, *P. indica* covers the entire petri plate, whereas, in the presence of *B. cepacia* LA3, the growth of *P. indica* was completely restricted. A change in the metabolome of the fungal biomass, as influenced by the bacterial metabolites, was investigated using high-resolution mass spectrometry. To enable us investigate a wide range of small molecular weight components, positive electro-spray ionization Fourier transform ion cyclotron resonance mass spectrometry with direct ion infusion (ESI+-FT-ICR-MS) at broad mass range was applied. Owing to the high resolution (100,000–500,000) and high mass accuracy (<0.5 ppm at *m/z* range of 150–1,000), 3,000–6,000 peaks were detected from the extract of the biomass. The peaks then were annotated to the metabolites listed in KEGG database applying online software (http://mips.gsf.de/proj/mbx/~masstrix/) that gives an overview of the possible presented and detectable metabolites within the characterized pathways and could be used for non-targeted metabolome screening, which, however, cannot differentiate the isomers. Since only a few fungal species have been investigated in this manner and *P. indica* has not been characterized in the KEGG database, a model organism had to be selected. The highest matches within the pathways were found, when *S. cerevisiae* was used as a model organism. The metabolome pattern of the methanol extract did not show dependency on the age of the *P. indica* biomass. Since there was no physical contact between the bacteria and the fungal hyphae, the inhibiting effect was caused by the excreted metabolite(s) of *B. cepacia* [51].

The number and type of metabolites within the pathways present in *S. cerevisiae* were compared with the metabolites in the extract of fungal biomass grown with and without the influence of *B. cepacia* LA3. Out of 110 pathways, 42 were further investigated, since these had more than three annotated metabolites. Changes in the number of annotated metabolites were observed in several pathways, but in some of them, like the phenylalanine metabolism and biosynthesis or sphingolipid metabolism, the metabolites were not influenced by the interaction with *B. cepacia* LA3.

A decrease in the annotated metabolites of the pentose phosphate and glycolysis was observed in the treated fungi compared with the control indicating a reduction in the activities of these pathways. In contrast, the number of annotated metabolites in the ubiquinone biosynthesis, limonene and pimene degradation, and folate biosynthesis greatly increased under the influence of the inhibitory bacterium.

The diffusible inhibitory metabolites of the *B. cepacia* LA3 exerted a complex influence on the metabolomic pathways of *P. indica*. Therefore, a mixture of small molecular weight and bioactive components were taken to compare the pathway differences of inhibited growth of the fungus. Saponin was selected as a model component mixture produced by plants. It is a complex mixture of biomolecules like steroids and terpenes having surfactant and inhibitory activity. The growth of *P. indica* grown in medium containing saponin was clearly inhibited, and the suppression was concentration dependent. The suppression of fungal growth was dependent on the concentration of the saponin in the range from 0.1 to 1.0 %.

Mechanism of Microbial Interactions

Several mechanisms, like the production of siderophores, antifungal metabolites, HCN, ammonia and lytic enzymes like chitinases and β-1,3-glucanases by rhizobacterial isolates, have been implicated in suppression of fungal growth [34]. The differential response of *P. indica* to different rhizobacteria leads us to postulate the existence of an ecological balance among the microbial communities in the rhizosphere/rhizoplane (mycorrhizosphere), which allows diverse microbial functional groups to coexist and share common resources.

The lower diameter of hypha in the *P. fluorescens* WS5-treated sample suggests the fungistatic nature of the 'inhibitory compound'. Spectrofluorimetry, gas chromatography data and mass spectra fragmentation patterns show the *m/z* value to be 416 suggesting thereby that one of the inhibitory substances could be pyoverdine (and its derivatives)—a potent siderophore [9, 14, 15]. Two contrasting observations, i.e. differential response of *P. indica* to the presence of rhizobacteria and its ability to survive in soil and colonize plant roots, led to the hypothesis that microbial communities interact through diffusible metabolites to counteract the inhibitory or stimulatory factor(s), thereby maintaining the delicate balance between diverse soil microorganisms. Concerning the inhibitory effects, antibiotics and lipopeptides produced and excreted by many biocontrol active rhizobacteria were shown to be responsible for the observed inhibition of *P. indica* by the plant growth-enhancing inoculant *B. amyloliquefaciens* FZB42 and the biocontrol rhizobacterium *Ps. fluorescens* SS101. It was clear from metabolome analysis that glycolysis and the pentose phosphate pathways were deactivated in the treated fungus compared with the control. Since both pathways play important roles in the energy maintenance, a decreased number of metabolites in the pathways responsible for energy production, and in consequence fungal growth, were greatly reduced. To

investigate if similar mixture has identical influence on the growth of the *P. indica*, saponine was used. The saponine treatment at amounts of 0.1–1 % suppressed the growth of the fungus that—according to the authors' knowledge—has not been reported before.

Genes Involved in Nutrient Uptake and Metabolism

The endophytic interaction of *P. indica* with plant roots is accompanied by an enormous acquisition of nitrogen and phosphorous from the environment [39, 56]. The growth promotion in *Arabidopsis* and tobacco seedlings by the fungus is attributed to enhanced nitrate uptake and the expression of the genes for nitrate reductase (*Nia2*) and the starch-degrading enzyme glucan-water dikinase (*SEX1*) in roots and shoots [39]. A very high activity of NADH-dependent nitrate reductase in the colonized roots results in a massive transfer of nitrogen into the aerial part of the seedlings [21].

Piriformospora indica-responsive *Nia2*, *SEX1*, and *2-nitropropane dioxygenase* genes are also upregulated in the colonized roots. *P. indica* also stimulates the expression of the *β*-glucuronidase gene (*uidA*) gene under the control of the *Arabidopsis* nitrate reductase (*Nia2*) promoter in transgenic tobacco seedlings. Therefore, the growth-promoting effect initiated by *P. indica* is accompanied by a co-regulated stimulation of enzymes involved in nitrate and starch metabolisms [39].

Uptake and transport of phosphorous, with diverse regulatory, structural, and energy transfer roles, are also stimulated by the fungus in the colonized roots of maize [56]. Recent studies have also shown that the sulphur metabolism is stimulated by the fungus. Genes which code for several plastid-localized enzymes required for sulphate reduction are upregulated by *P. indica* in *Arabidopsis* roots, and gene inactivation studies confirmed that they are required for the benefits to the plants.

Stimulatory Factors

The analytical HPLC separation of *P. indica* culture supernatant showed seven peaks in the hyphae and one main peak in the culture filtrate. Preparative HPLC analysis of hyphal and culture filtrate showed a major peak identified as benzoic acid. The function of this compound is not clear. Compounds identical to benzoic acid and its analogues (benzoic acid, a-hydroxybenzoic acid, 3-4 di-hydroxybenzoic acid, vanillic acid, cinnamic acid, p-coumaric acid, caffeic acid and ferulic acid) did not show any stimulation on the plants tested. The nature of the stimulatory factors which promote the plant growth is not yet known [2, 29, 49].

Nutrient Transport

Experiments using ^{32}P have shown that *P. indica* plays an important role for the acquisition of 'P' by the roots especially in the arid and semi-arid regions [50]. Cloning and functional analysis of a gene encoding a phosphate transporter (*PiPT*) from this root endophyte is reported [56]. The *PiPT* polypeptide belongs to the major facilitator super family (MFS) and exhibits 12 transmembrane helices divided into two halves that are connected by a large hydrophilic loop in the middle. The function of the protein encoded by *PiPT* was confirmed by complementation of a yeast phosphate transporter mutant. *PiPT* belongs to high affinity phosphate transporter family (Pht1). To understand the physiological role of *PiPT*, knockdown (KD) transformants of the gene were prepared using electroporation and RNA interference. KD transformants transported a significantly lower amount of phosphate to the host plant than wild-type *P. indica*. Higher amounts of phosphate were found in plants colonized with wild-type *P. indica* than that of non-colonized and plants colonized with KD-*PiPT P. indica*. These observations suggest that s*PiPT* is actively involved in the phosphate transportation and in turn *P. indica* helps to improve the nutritional status of the host plant [56].

Regulation of Genes Involved in Resistance to Abiotic Stress

Drought Stress

Piriformospora indica effectively helps plants to overcome abiotic stress like drought in *Arabidopsis thaliana* and Chinese cabbage (*Brassica campestris* L. ssp. *Chinensis*) [40, 44, 47]. *P. indica*-colonized *A. thaliana* plants exposed to drought showed increased drought tolerance and continued to grow, whereas in uncolonized controls, growth was inhibited [40, 41, 47]. Colonized plants had higher chlorophyll content and increased photosynthetic efficiency under drought-stress compared to the uncolonized plants [40, 43]. Different genes involved in quite diverse processes of drought and other stress responses, i.e. response to dehydration (RD29A), early response to dehydration (ERD1), phospholipase D*δ* (PLD), the transcription factor gene (ANAC072), dehydration-response element-binding protein (DREB2A), salt- and drought-induced ring finger (SDIR1), calcineurin B-like protein (CBL1), CBL-interacting protein kinase (CIPK3), were rapidly upregulated to a higher extent in the leaves of *P. indica*-colonized *Arabidopsis* seedlings compared to the controls [40]. These genes are involved in quite different cellular processes, starting from phospholipid metabolism at the plasma membrane (PLD*δ*), via cytoplasmic signalling through

CBL1/CIPK3, control of gene expression in the nucleus (HAT, DREB2A and ANAC072) to cytoplasmic functions associated with RD29A and protein degradation at the endomembrane system (SDIR1) as well as in the plastids (ERD1). The expressions of two ascorbate reductase genes, viz. *MDAR2* and *DHAR5*, are upregulated in the roots and shoots of colonized *Arabidopsis* seedlings exposed to drought [47]. Monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) are two enzymes of the ascorbate–glutathione cycle that maintain ascorbate, a major antioxidant and radical oxygen scavenger, in its reduced state. Growth and seed production were not promoted in the colonized *mdar2* and *dhar5* mutants indicating that MDAR2 and DHAR5 are crucial for producing sufficient ascorbate to maintain the interaction in a mutualistic state [47]. Thus, *P. indica* confers drought tolerance to *Arabidopsis* by priming the aerial parts of the plant for an early and high expression of drought-related genes [40, 47].

When *P. indica*-colonized Chinese cabbage plants were exposed to polyethylene glycol to mimic drought stress, the activities of antioxidant enzymes, viz., peroxidases, catalases and superoxide dismutases in the leaves were significantly upregulated within 24 h [44]. The fungus could retard accumulation of malondialdeyde—a biomarker of oxidative stress, the drought-induced decline in the photosynthetic efficiency and the degradation of chlorophylls and thylakoid proteins in the colonized plants [44]. The expression levels of the drought-related genes *DREB2A*, *CBL1*, *ANAC072* and *RD29A* were upregulated in the drought-stressed leaves of colonized plants. Furthermore, the *CAS* mRNA level for the thylakoid membrane associated to Ca^{2+}-sensing regulator (CAS) and the amount of the CAS protein increased in the colonized plants compared with the controls [44]. Therefore, the drought tolerance in Chinese cabbage is associated with the activation of antioxidant enzyme activities, the upregulation of mRNA levels for drought-related proteins, and rapid accumulation of the plastid-localized CAS protein in the leaves of the colonized plants [25, 44].

Low Temperature Tolerance

Influence of *P. indica* on seed germination under extreme low temperatures was studied in 12 leafy vegetable plants (Table 2), at Leh–Ladakh (DIHAR, DRDO laboratory), India at an altitude of 3,500 m. Within 25 days of plating, 100 % germination was obtained in the seeds of cabbage, endive, Swisschord (Palak), Swisschord (Red), radish and onion treated with *P. indica* (Table 2), whereas, not a single seed germinated in untreated control. The plants treated with *P. indica* grew better compared with the untreated control plants, and after about 3 months' growth in microplots, significant increases in the growth of cabbage, cauliflower and beetroot were recorded (Fig. 4) [26, 50]. It is important to note that *P. indica*, which is commonly associated with plants growing under extreme hot conditions of Thar desert of western Rajasthan, India also interacted with plants at high altitudes demonstrating unique features which have not been recorded so far (See Patent No. 709/DEL/2011 dated 15th March 2011).

Table 2 Percentage germination of seeds of vegetables commonly grown at high altitudes in Leh, Ladakh

Hosts	Days after sowing	% Increase in germination
Cabbage	25	100
Endive	25	100
Swisschord (Palak)	25	100
Swisschord (Red)	25	100
Radish	25	100
Onion	25	100
Carrot	21	84
Cauliflower	21	84
Beetroot	20	80
Peas	15	60
Snowpea	12	48

Salt and Nutrient Stress

It has been shown that this fungus (*P. indica*) can grow even under conditions of high salt concentration of 219.14 mM, while its growth was inhibited at salt concentration 438.27 mM (Fig. 5) [8, 25]. *P. indica* promoted stress tolerance (salt and nutrient) on the co-cultivated tomato (*Solanum lycopersicum* 'Roma') compared with the control through the activation of antioxidant metabolism, which leads to the accumulation of ascorbate (vitamin C). Tomato fruits borne on the *P. indica*-treated plants maintain lycopene content independent of the growing conditions. Gosal et al. [17] demonstrated that biotization of micropropagated *Chlorophytum borivilianum* with *P. indica* improves plantlet survival rate: 'P' content, the most important nutrient acquisition. Cu, Fe, Zn and Mn uptakes are also improved in the plantlets treated with *P. indica.*

Treatment of micropropagated sugarcane plantlets with *P. indica* improved their survival and performance (Table 3). Colonization of inoculated sugarcane roots was extended to 91.8 % in sugarcane cv. CoJ 83 and 92.5 % in cv. CoJ 88 after 4 weeks of growth in the greenhouse. Cane yield and yield components (tillering and cane height) in biohardened field of cv. CoJ 88 were significantly higher than both the non-inoculated micropropagated and non-inoculated conventionally propagated sugarcane. Similar observations were made in the ratoon crop too. Iron deficiency was observed in the majority of un-inoculated

Fig. 4 *Piriformospora indica* promotes plant growth (under extreme temperature stress at high altitude). **a** Cabbage; **b** beetroot (*left* control; *right P. indica*-treated plants after nearly three months of planting). Interactive experiments were conducted at the Defence Institute of Higher Altitude Research (DIHAR), DRDO, Leh, Ladakh, India at an altitude of 3,300 m

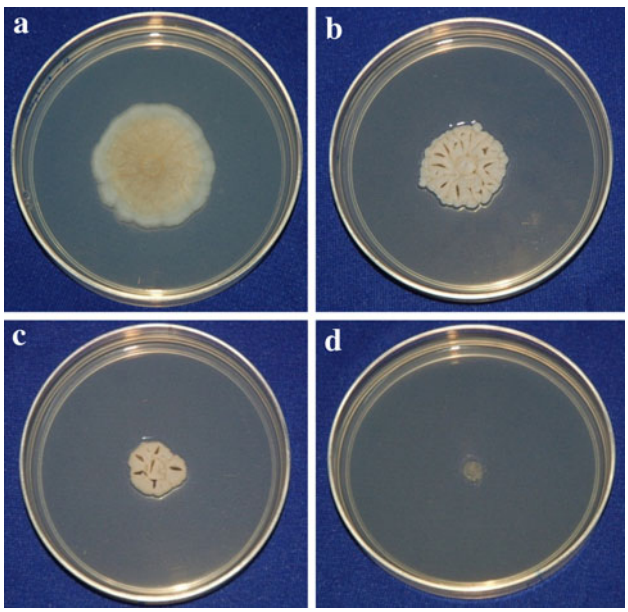

Fig. 5 Growth of *P. indica* on Modified Melin and Norkrans medium in Control and high NaCl concentrations; **a** 0.43 mM (Control), **b** 109.57 mM, **c** 219.14 mM, **d** 438.27 mM (*Source*: Chordia et al. [8])

ratoon crop plants, but this was not the case with the inoculated ones where the uptake of both Fe and Cu was promoted.

Interaction with Plants

Impact of Fungal Biomass

Cereals

Piriformospora indica increases nutrient uptake, allows plants to survive under drought, high- and low-temperature and salt stresses, confers systemic resistance to toxins, heavy metal ions and pathogenic organisms, and stimulates growth and seed production. Several other authors [1, 12, 17, 24, 27–29, 35, 37, 38, 40, 41, 44–49, 52, 53] have reported that the fungus improves the growth and overall biomass production of diverse hosts. A total number of approximately 150 plant species are reported to interact with *P. indica* including agricultural, horticultural, medicinal and other important plants. *Oryza sativa*, *Zea mays*, *Tridax procumbans*, *Nicotiana tabacum*, *Arabidopsis thaliana* and *Brassica oleracea* var *capitata* plants have been shown to have improved seed germination and an increase in seed formation.

Many plants, viz. *Oryza sativa*, *Zea mays*, *Phaseolus vulgaris*, *Tridax procumbans*, *Abrus precatorius*, *Solanum nigrum*, *Brassica oleracea* var *capitata*, *Brassica nigra*, *Nicotiana tabbacum*, *Saccharum officinarum*, *Lagenaria* sp. and *Spinacea oleracea*, have shown better plant growth

Table 3 Interaction of *P. indica* with *Saccharum officinarum* (var. CoJ 88), in a field trial at Punjab Agricultural University, Ludhiana (courtesy, Dr. S. K. Gosal)

Treatments	Tiller number/ clump	Cane number/ clump	Cane height (cm)	Cane girth (cm)	Brix (Sugar content)	Weight per clump (kg)	Weight per plot (kg)
Control	9.27	8.10	179	2.22	18.35	6.50	122.2
P. indica	17.2	15.90	191	2.21	21.40	7.34	138.3
CD (5 %)	2.59	2.51	NS	NS	1.99	NS	2.39

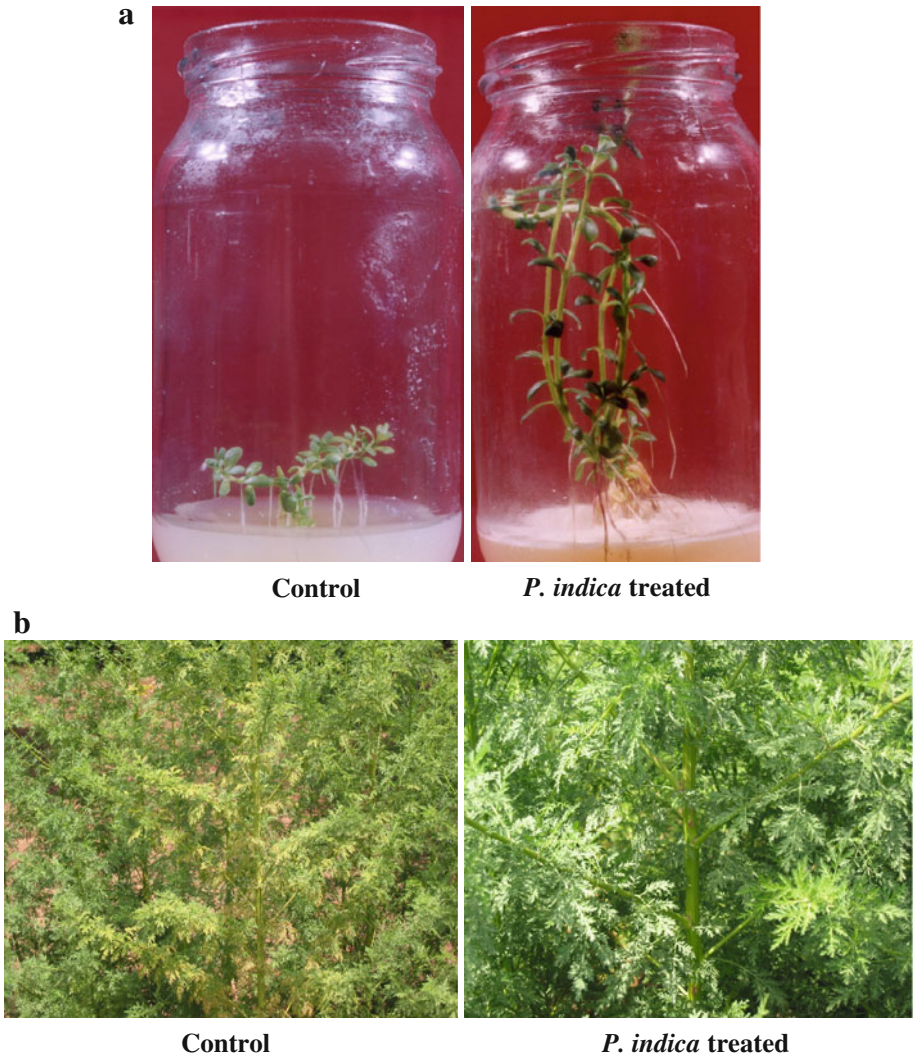

Fig. 6 *P. indica* promotes growth of medicinally important plants under control and field conditions. **a** *Bacopa monniera* seedlings showing better growth in medium co-inoculated with *P. indica* (*right flask*) [32]; **b** *Artemisia annua* showed good growth in field plots treated with *P. Indica* (*right*) at Panchmarhi, Madhya Pradesh

with increased biomass production [31]. *P. indica*-colonized roots show a higher development compared to the control plants at the initial stages of growth, as suggested by earlier expression of developmentally regulated genes [53]. Kumar et al. [24] have shown that maize roots inoculated with *P. indica* show enhanced growth response by the colonization of the exterior root cortex. This fungus also promotes the growth of several tropical legumes (*Cicer arietinum*, *Phaseolus aureus*, *P. mungo*, *Pisum sativum* and *Glycine max*) [49, 50]

Medicinal Plants

Piriformospora indica interacts closely with medicinal plants *Artemisia annua*, *Bacopa monniera*, *Abrus precatorius*, *Stevia rebaudiana*, *Linum album*, *Trigonella foenum-graecum*, *Coleus forskohlii*, *Spilanthes calva*, *Withania somniferra*, *Chlorophytum borivilianu*, *Tridax procumbens*, *Curcuma longa*, *Podophyllum peltatum*, *Azatarichta indica*, *Foeniculum vulgare*, *Oscimum sanctum* [10, 11, 16, 27]. Some of the important medicinal plants which have been

reported to beneficially interact with the fungus are shown in Fig. 6. The secondary metabolite contents of all these medicinal plants are found to increase several folds because of their interaction with *P. indica.* The fungus remained neutral and did not colonize the roots of Naga chilli (*Capsicum chinense*), which was considered as the hottest chilly of the world (557,000 Scoville units, http.//chilly.in/Indian_chilli_varieties.htm) primarily growing in the North Eastern region of India [50]. Root colonization resulted in early flowering in the plants tested, like in *Coleus forskohlii*, bottle gourd and *Nicotiana tabacum* (Fig. 7).

In another study, co-cultivation of live fungal cells with hairy roots of medicinal plant *Linum album* reduced the growth of hairy roots. Despite reduction in hairy root biomass, an enhancement in lignin content was observed. The hairy root cultures co-cultivated with 1–5 g/l fungal biomass at days 10–13, all achieved a higher podophyllotoxin (PT) and 6-methoxypodophyllotoxin (6-MPT) content (mg/g) in the roots other than in the fungus-free control culture. The highest increase in PT content (8.48 mg/g) and 6-MPT content (3.78 mg/g) was obtained when a fungal concentration of 2.0 g/l was added to a growing hairy root cultures of *L. album* on 12th day, i.e. for exposure time of 48 h. The same fungal concentration for the same duration resulted in maximum improvement by 2.1-fold in PT concentration and 2.5-fold in 6-MPT concentrations, respectively [6, 36].

Impact of Fungal Culture Filtrate

The culture filtrate of the mycelium contains fungal exudates, minerals, hormones, enzymes, proteins, etc. [2, 49, 52]. In vitro experiments have shown that even very small amounts (2.5 ml/l of medium) of culture filtrate are sufficient to promote root and shoot growth. In vitro interaction experiments were tested for *Triticum aestivum*, *Cicer arietinum*, *Phaseolus vulgaris* (Fig. 8a), *Brassica campestris* and *Broccoli.* The culture filtrate is effective in breaking the dormancy, seed germination and enhancement of the seedlings. This study was repeated in green house, employing pot experiments: 15-day-old seedlings of *Zea mays*, *Brassica oleracea* and *Helianthus annuus* were transferred to plastic pots containing vermiculite (autoclaved) and sand (acid washed) in the ratio of 3:1. An amount of 15 ml freshly eluted *P. indica* culture filtrate was applied to each pot containing one kilogram of substratum. Equal amount of sterile Hill–Käefer medium was added to control. Increases in the root and shoot lengths and plant biomass were observed in the *P. indica*-treated hosts (Fig. 8b) [5].

Fig. 7 *Piriformospora indica* is shown to induce early flowering in *Coleus forskohlii*; the plant on the *right* was treated with *P. indica*

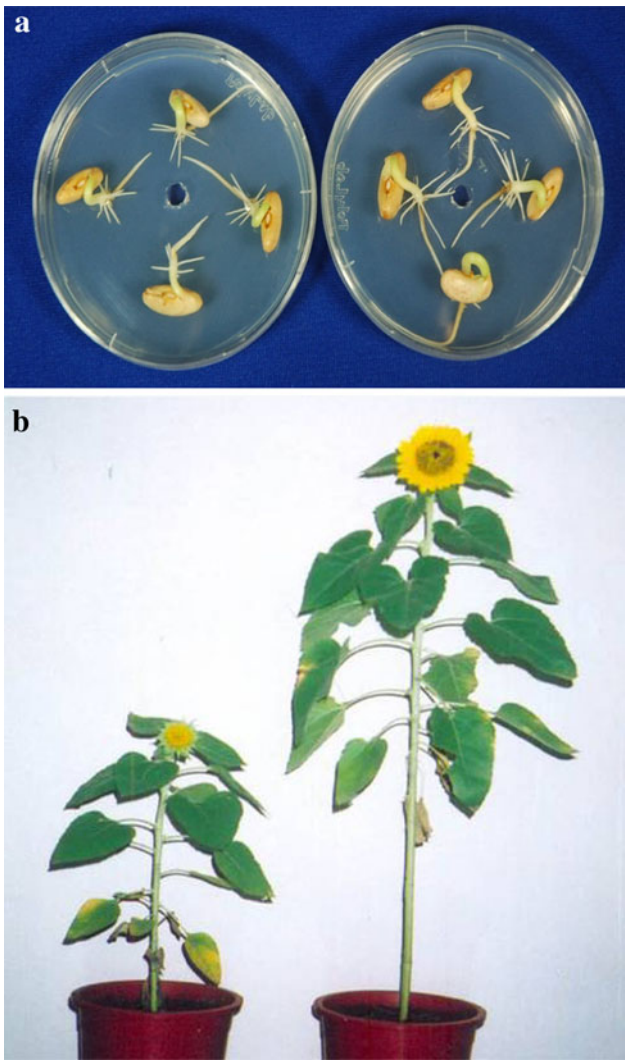

Fig. 8 Promotion of plant growth by *P. indica* culture filtrates. **a** Improvement in the germination of *Phaseolus vulgaris* seeds treated by placing 15-µl sterile culture filtrate of *P. indica* in the central well (*right*), compared with sterile water control (*left*). **b** Improved growth of *H. annuus* plant treated with 15 ml of sterile *P. indica* culture filtrate (*right*) compared with the control plant (*left*)

Conclusion and Future Prospective

Piriformospora indica is a well-established symbiont, which benefits plant growth and increases the resistance against pathogens in a broad range of host plants. This study has special significance as the fungus is being exploited for biotechnological applications in the area of agriculture, forestry, arboriculture and flori-horticulture in field, as well as hydroponics cultivation of several vegetables and aromatic hosts. For a possible combined application of *P. indica* with plant growth-promoting rhizobacteria, it has to be tested beforehand so that an inhibitory effect of these rhizobacteria on *P. indica* does not occur. Concerning its beneficial nature, *P. indica* and allied members have considerable potential as bio-control agents and plant-growth promoters. Special efforts should therefore be made to define the molecular and biochemical bases of symbioses and their physiological effects on crop plants, as it is a prerequisite for the introduction of the fungus to different cropping systems. *P. indica* has been extremely valuable in understanding the orchestration of root innate immunity. In particular, the *Arabidopsis–P. indica* system allows for a comparison between root and leaf innate immunities. Further, one may gain an insight into how roots discriminate between pathogens and mutualists. Considering its immune suppressive capacities, *P. indica* might also represent a unique tool to uncover the points of plant innate immunity. The recently accomplished annotation of the *P. indica* genome will undoubtedly support all research efforts related to *P. indica*, but also to other mutualistic plant-colonizing fungi. Moreover, we have just established a stable barley root transformation system that allows the fast and robust analysis of *P. indica* protein functions in all aspects of root development, as well as abiotic stress tolerance and biotic interactions in crop plants.

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