Chapter 17
Current Progress on Endophytic Microbial Dynamics on *Dendrobium* Plants

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17.1 Introduction

From the start of human civilization, medicinal plant parts, extracts, phytochemicals, and conventional botanical mixtures have been widely used for the healing of diverse human diseases (Dey and Mukherjee 2018). The orchids of the Orchidaceae family possibly originated 120 million years ago. It is the largest major family among angiosperms; further, the majority of this family is composed of highly evolved flowering plants, with approximately 25,000–35,000 species in 750–900 genera (Lam et al. 2015). *Dendrobium* is recognized as Shihu, broadly dispersed throughout China with 78 species, and approximately 30 species (of these 78 species) have been used as a chief medicinal and efficient food over many centuries, such as tea drinks or soup ingredients (Deng et al. 2017). *Dendrobium* plants are either lithophytic or epiphytic, frequently with aerial roots and buds (Ng et al.

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2012). Most *Dendrobium* plants are cross-pollinated, and within the same plant species, the maturity periods of pollen and stigma are frequently altered for additional ornamental cross-pollination (Yan et al. 2015). Numerous Orchidaceae, especially the stems of *Dendrobium* species, are widely used to supply remedial benefits through enhancing saliva secretion, reducing fever, and immunity enhancement (Jiangsu New Medicinal University 1986). This remedy is also useful for tonicity of the stomach, maintaining and enhancing the body fluid production level (Cui et al. 2014). Many bioactive parts of *Dendrobium* are well documented including bibenzyls, phenanthrenes, sesquiterpenes, and alkaloids (Ng et al. 2012; Zhang et al. 2017). It is an essential long-established Chinese medicine with efficient alkaloids with numerous pharmacological uses including immune regulation, antioxidation, antitumor, bacteriostasis, and hypoglycemia functions.

Dendrobium is typically propagated by extrication backbulbs and keikies, or by vegetative cuttings, but these are extremely slow and difficult methods that result in the survival of only a few propagules (da Silva et al. 2017). In fact, polysaccharides are one of the abundant components in *Dendrobium* species, and immunostimulatory action is one of the most vital biological actions of these polysaccharides (Deng et al. 2017; Yang et al. 2017).

Similar to root-inhabiting bacteria, endophytic microbes have mechanisms by which they support plant health and growth (Yi et al. 2013). Endophytic bacteria inhabit within the host plant tissue without unfavorably harming it. The impact of their bioactive compounds has been verified as positive in a wide variety of crops (Ryan et al. 2008). These compounds may contribute directly to plant growth by increased nutrient availability, fixation of biological nitrogen, and phytohormone production (Kim et al. 2011). Ultimately, they may also indirectly decrease microbial numbers that are dangerous to plant growth, and behave as biological control agents through antibiosis, competition, or systemic resistance stimulation (Yi et al. 2013). The quest for more capable endophytic microbial strains has been stimulated by the idea that these organisms generate a plethora of bioactive products (Wu et al. 2016).

Cultivable endophyte diversity occurred not only in the multiplicity of plant species occupied but also in the numerous taxa concerned, with the majority being linked with predominant organisms found in the soil (Mehnaz et al. 2009). Some bacteria such as *Bacillus pumilus* INR7 are distinctive examples for a biocontrol artefact that has been established using the ability of a single endophytic bacteria to provoke plant growth promotion (PGP) and induced systemic resistance (ISR) (Jeong et al. 2014). Mycorrhization could be an effective alternate way to recover the rooting and existence of *Dendrobium*. Photoautotrophic *Dendrobium* culture is a still unexplored aspect but could be a way to improve the efficacy of acclimatization events (da Silva et al. 2017).

In *Dendrobium* plant organs, cell culture technology has established countless approaches for making plant-specific beneficial metabolites, together with pharmaceuticals, acceptable chemicals, and coloring mediators. Efforts have been completed for the formation of cells with improved root cultures for assembly of value-added compounds (Cui et al. 2011). Protocorm, embryo, root, and shoot cultures are regularly applied for the production of industrial compounds (Naik et al. 2011). Bioreactor usage for large-scale production of cells and extrinsic roots has converted these into feasible cells for metabolite production (Cui et al. 2011), although numerous chemical as well as physical aspects need to be enhanced for effective metabolite production through bioreactor cultures (Lee et al. 2011; Lee and Paek 2012).

Now, denrobine, a major component of *Dendrobium* species, is increasingly drawing attention for its wide applications in healthcare, and the demand is ever increasing, but the wild resources of *Dendrobium* species cannot meet the market requirements. Thus, artificial cultivation of *Dendrobium* plants is extremely urgent (Li et al. 2017a, b, c).

17.2 Microbial Dynamics of *Dendrobium* Plants

The conventional approaches are not appropriate for preparing a bulky quality planting material. Several orchids can be spread sexually through seeds, but *Dendrobium* seeds are deficient in endosperm and nutritious substances, and it is usually difficult to produce these as wide-ranging plants in nature because seed germination requires symbiotic endophytic microbes. Whenever a new position of biological diversity is revealed and accessed, novel natural products are established (Fig. 17.1). The understanding that there are bulky, and typically unexplored, endophytic microbe clusters living inside the developed plants (endophytic fungi) led to

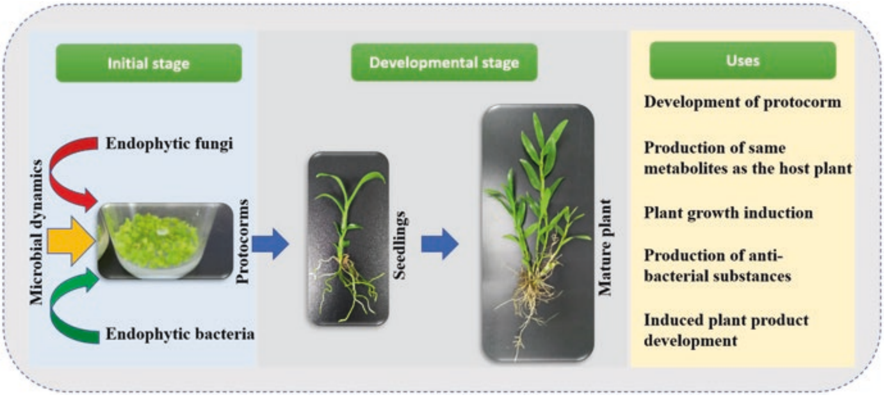

Fig. 17.1 Microbial dynamics of the *Dendrobium* plant for growth development and natural products

intensive discovery trials in both industrial and academic laboratories (Winarto et al. 2013).

17.2.1 Endophytic Fungi

Endophytic fungi associated with plants are fungal microbes that employ all or part of their development inhabiting healthy tissues of their plant hosts inter- or intracellularly, characteristically causing no superficial disease signs. These organizationally varied molecules have possible therapeutic importance, which has increased common interest in the transmission of endophytic fungi for the detection of novel metabolites and also specific novel antibiotics. The natural products that are typically "to blame" for the identification of endophytic fungi of plants as a vital source of accepted bioactive products include paclitaxel (taxol) because of the recognition of the taxol-forming fungal endophyte *Taxomyces andreanae* in 1993 (Stierle et al. 1993).

The fungal mycorrhiza has some incomparable characteristics in the life sequence of orchid plants (Singh et al. 2017). In the natural landscape, orchid linkage with many mycorrhizal fungi is vital for seed development because orchids lack endosperm and their seedling development requires nutrients from an external source. Mycorrhiza have significant effects on the development of the plant life cycle, and vegetative and propagative evolution (Hoysted et al. 2018). Sarsaiya et al. (2019a) have described a comprehensive evaluation of endophytic fungi for novel and critical insights on physiological, genomics, and functional analyses that provided novel information useful to overcome the growing challenges for *Dendrobium* development. On the other hand, Sarsaiya et al. (2019b) have reported for the first time that *Trichoderma longibrachiatum* caused leaf black circular spots on *Dendrobium nobile* in Guizhou Province, China.

Fungi allied with orchids are mostly contained in the genus *Rhizoctonia* (class Hymenomycetes, subdivision Basidiomycota). Nine orchid species that breed in Puerto Rico have a close association with as many as 108 *Rhizoctonia*-like fungi that include *Tulasnella*, *Thanatephorus*, and *Ceratobasidium* (Agustini et al. 2016). As Taylor and Bruns (1997) and Taylor et al. (2004) showed, 17 to 22 fungal species allied with photosynthetic orchids are in the Russulaceae family. Investigation on *Limodorum abortivum*, a natural orchid budding in Mediterranean areas, exhibited its relationship with fungi of the family Russulaceae (Gurlanda et al. 2006). Characterization of the orchid fungal mycorrhiza is very important when reviewing their association. Morphological characterization of mycorrhizal pure culture by means of special characters such as form, color, and colony pattern can only provide identification at the genus level; however, species-level identification is more available with the support of molecular identification. These molecular methods are simple because the DNA sequence that is required is not very complex. The small sequences such as internal transcribed spacer (ITS) among the small subunit (SSU) as well as large subunit (LSU) recombinant DNA can be used for species identification (Agustini et al. 2016).

Agustini et al. (2009) described that numerous orchid species in Papua, together with the *Calanthe* species of orchids, have symbiotic association with fungal mycorrhiza (Agustini et al. 2016). Orchidaceae genera are exceptional in numerous adaption characters such as the velamen root, symbiotic linking between fungi, and specific flowers that evolved strictly with insect pollinators (Hossain et al. 2013). The orchid roots together are attached with the aerial roots to the substrate; the substrate roots are linked with numerous endotrophic fungi (da Silva et al. 2015). Both nonmycorrhizal and mycorrhizal endophytes cooperate with their plant host in several biological actions. The fungi offer advantages to the orchid plants in various ecological aspects such as in low light (tuberous species and terrestrial rhizomatous species), in low-nutrient soil or spatial distribution, bark, development of siblings and seedlings, and on morphology (Rasmussen 1995). The association of orchids with mycorrhiza is a symbiosis type of cooperation wherein both the fungi and the plant are mutually promoted by each other; therefore, the relationship is considered mutualism. The mycorrhizae increase the plant contact with soil properties such as mineral ions and water and can support the continued growth of orchids in a dormant phase for many years. The fungi have multifunctional ability to produce wide-ranging plant growth development substances such as auxin (indole-3-acetic acid, IAA), gibberellic acid (GA3), abscisic acid, zeatin riboside, and zeatin (Table 17.1) (Liu et al. 2010; Parthibhan et al. 2017).

The various nonmycorrhizal fungi of orchids, also named endophytes, have also been purified in orchids from numerous tissues, including leaf, stem color, stem, rhizome, tubers, mature bulb, and even also from roots (Sarsaiya et al. 2019; Lin et al. 2020). Their mycelia have been described to secrete numerous plant growth-encouraging substances such as GA, auxin (IAA), and the vitamins B6, B2, and folic acid (Table 17.2) (Maor et al. 2004). Therefore, their symbiotic association for in vitro and in vivo seed germination and seedling development is described to support germination as well as fresh weight (Yang et al. 2008), although proven indications for the nutrient streams from fungal organisms to orchids are known only for carbon and nitrogen compounds (Stöckel et al. 2014); the transfer ensues either from the breakdown pelotons or from both living and breakdown forms of pelotons (Kuga et al. 2014). Because the symbiont has a strong impact on orchid growth, it is very important to study their fungal symbiotic relationships to develop new strategies for orchid conservation and better exploitation of their medicinal compounds (Parthibhan et al. 2017). The findings of Chen et al. (2011) showed that all ten plant species of *Dendrobium* were responsible for harboring endophytic fungi. The endophyte taxa *Acremonium*, *Alternaria*, *Ampelomyces*, *Cladosporium*, *Colletotrichum*, *Fusarium*, and *Verticillium* originated in most *Dendrobium* species.

The xylariaceous fungi occur frequently among the medically important *Dendrobium* plants, but their biological functions have remained uncertain up to the present. Rodriguez et al. (2009) revealed that xylariaceous fungi in the living world might be mutualistic with their plant host, even though the *Xylaria* association of mycorrhizal fungi in *Dendrobium* and other orchid plants has yet to be established. Furthermore, endophytic fungi show an imperative role in variable secondary metabolite production in their host orchid plants and may increase the concentrations of complete alkaloids and polysaccharides in therapeutically important

Table 17.1 Diversity of endophytic microbes with abundance and frequency on *Dendrobium* plants

Endophytic microbial taxa	Abundance/ frequency	Source	Reference
Fusarium oxysporum	22	*Dendrobiium officinale:* roots, stems	Jin et al. (2017)
Xylaria cubensis	21	*D. officinale:* roots, stems, leaves	Jin et al. (2017)
Colletotrichum spp.	12	*D. officinale:* roots, stems, leaves	Jin et al. (2017)
Pestalotiopsis spp.	07	*D. officinale:* roots, stems	Jin et al. (2017)
Alternaria spp.	06	*D. officinale:* roots, stems, leaves	Jin et al. (2017)
Aspergillus niger	06	*D. officinale:* stems, leaves	Jin et al. (2017)
Aspergillus flavus	06	*D. officinale:* roots, stems	Jin et al. (2017)
Trichoderma atroviride	05	*D. officinale:* roots, stems, leaves	Jin et al. (2017)
Cochliobolus spp.	03	*D. officinale:* roots, stems	Jin et al. (2017)
Cystobasidium slooffiae	02	*D. officinale:* roots	Jin et al. (2017)
Aureobasidium pullulans	02	*D. officinale:* roots	Jin et al. (2017)
Epicoccum sorghinum	02	*D. officinale: r*oots, stems	Jin et al. (2017)
Ceratobasidium spp.	NR	*D. lancifolium*	Agustini et al. (2016)
Guignardia endophyllicola	28	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Colletotrichum gloeosporioides	13	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Cladosporium sphaerospermum	02	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Fusarium solani	02	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Xylohypha spp.	02	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Colletotrichum spp.	01	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Curvularia brachyspora	01	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Fusarium nivale	01	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Pestalotiopsis spp.	01	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Scolecobasidium spp.	01	*D. crumenatum:* roots, stems	Mangunwardoyo et al. (2011)
Westerdikella spp.	01	*D. crumenatum: Roots, stems*	Mangunwardoyo et al. (2011)
Pestalotiopsis vismiae	01	*D. nobile:* stems	Yuan et al. (2009)
Colletotrichum species	10	*D. nobile:* stems, leaves, roots	Yuan et al. (2009)

(continued)

Table 17.1 (continued)

Endophytic microbial taxa	Abundance/frequency	Source	Reference
Phomopsis amygdale	05	*D. nobile:* stems, roots	Yuan et al. (2009)
Phomopsis spp.	07	*D. nobile:* stems, roots	Yuan et al. (2009)
Clonostachys rosea	02	*D. nobile:* roots	Yuan et al. (2009)
Penicillium griseofulvum	07	*D. nobile:* stems, leaves, roots	Yuan et al. (2009)
Penicillium spp.	03	*D. nobile:* stems	Yuan et al. (2009)
Trichoderma chlorosporum	02	*D. nobile:* roots	Yuan et al. (2009)
Fusarium solani	02	*D. nobile:* roots	Yuan et al. (2009)
Fusarium proliferatum	06	*D. nobile:* stems, roots	Yuan et al. (2009)
Fusarium spp.	03	*D. nobile:* roots	Yuan et al. (2009)
Guignardia mangiferae	52	*D. nobile:* stems, leaves	Yuan et al. (2009)
Botryosphaeria spp.	02	*D. nobile:* stems	Yuan et al. (2009)
Xylaria spp.	25	*D. nobile:* stems, leaves, roots	Yuan et al. (2009)
Hypoxylon spp.	02	*D. nobile:* stems, leaves	Yuan et al. (2009)
Nemania spp.	01	*D. nobile:* stems	Yuan et al. (2009)
Rhizoctonia spp.	02	*D. nobile:* stems	Yuan et al. (2009)

Table 17.2 Effect of endophytic microbes on *Dendrobium* plant morphology and its development

Plant	Product	Microbes	Effect on plant morphology and development	Reference
Dendrobium nobile	Auxin (bioactive)	Bacterial species: *Azospirillum*, *Enterobacter*, *Streptomyces*, *Mycobacterium*, and *Bacillus pumilus*	Herb seed germination and its expansion	Tsavkelova et al. (2016)
D. friedericksianum	Not specified	Fungal species: *Trichosporiella multisporum*	Herb seed propagation	Khamchatra et al. (2016)

Dendrobium plants. Consequently, those secondary metabolites from xylariaceous taxa abundant in *Dendrobium* should be extensively discovered, and the association among the endophytic *Xylaria* strains and the eminence of *Dendrobium* as a medicine should be widely examined (Chen et al. 2013).

17.2.2 Endophytic Bacteria

Dendrobium plant-linked bacteria are generally known to have unquantified and often advantageous influences on plant growth because of nitrogen fixation, plant growth controllers, water uptake and mineral nutrition, and biosynthesis of bactericidal and fungicidal constituents, therefore decreasing the amount of pathogens in the herbs (Li et al. 2017a, b, c). Endophytic bacteria populations, as a rich source of natural compounds as well as phytochemicals by means of promising therapeutic and agricultural utilization, have invited much consideration from investigators worldwide (Ryan et al. 2008; Bhore et al. 2013; Brader et al. 2014). Plant growth-promoting (PGP) bacteria is a complex, well-established phenomenon that is often accomplished by numerous probiotic traits revealed by the related bacterium, such as antagonism against plant pathogens (Haas and Défago 2005). The genera *Alternaria, Botrytis, Verticillium, Fusarium, Rhizoctonia*, and *Pyricularia* are dominant plant pathogens that contaminate a wide variety of plants, as well as vegetables, several crops, and fruit, causing important economic losses (Khush and Jena 2009; Williamson et al. 2007). In some other investigations, 22 endophytic stains showed antagonistic action in at least single plant pathogen analysis, signifying that these cultures have the possibility to be applied as biofungicides. Many procedures have been planned for antibiosis reaction, which could be arbitrated by the discharge of nonspecific or specific microbial metabolites, extracellular biocatalysts, or the formation of siderophores and other substances (Arora et al. 2008). Approximately all the analyzed antagonistic strains were able to yield biocatalysts such as protease and siderophores. The consequent plant pathogen elimination by potential antagonistic strains could be comparatively attributed to protease and siderophore production (Wu et al. 2016).

17.2.3 Physiology of Fungi in the Growth Induction of Dendrobium

The epiphytic *Dendrobium* species are greatly valued for their medically important bioactive substances, although their endophytic fungal populations are comparatively less well known (Chen et al. 2011). In *Dendrobium nobile*, Hyphomycetes, Coelomycetes, and Ascomycetes occurred as the most dominant endophytic fungi, showing complex tissue specificity and accumulation in the plant leaves, and subsequently in the stem as well as roots (Yuan et al. 2009). Two of the root fungal endophytes, *Trichoderma chlorosporum* and *Clonostachys rosea*, were found to have advantages for plant acclimatization and represented possible biocontrol and growth advancement characteristics correspondingly. In *D. loddigesii*, *Acremonium* and *Fusarium* occurred as the most dominant fungal isolates, indicating high assemblage and establishment in roots and thereafter into the leaves along with stems where a few isolates expressed antimicrobial as well as plant growth advancement (Chen et al. 2010). In the same way, several *Dendrobium* herb varieties, such as *D. fimbriatum*, *D. chrysanthum*, *D. officinale*, *D. devonianum*, *D. nobile*, and *D. thyrsiflorum*, have been widely documented to link with varied endophytic fungi with a high grade of assemblage and specificity (Hajong et al. 2013). These investigations suggested that the diverse endophytic fungi screened from *Dendrobium* plant species could have diverse applications (Parthibhan et al. 2017).

Several investigations have been devoted to the orchid–fungal interface and manipulation of its valuable substances. The additional sugar supply as an energy source provided from the fungi supports the *Dendrobium* plants to metabolize their stored food reserves for growth and development (Sarsaiya et al. 2020a, b). After the purpose of the linkage is fulfilled, the orchid still maintains the linkage with similar or different or various endophyte fungi in the roots and other organs (Smith and Read 2008). The nonmycorrhizal orchid endophyte fungi have been known for their manifold ecological functions and bioactive abilities (Yuan et al. 2009), although most of the investigations have recognized only identifications and characterizations of the many fungal endophytes. Thus, the roles of these nonmycorrhizal fungi are currently largely unknown (Parthibhan et al. 2017).

In orchid plants, leaf and stem endophytic fungi are reported to depress the mycorrhizal fungi development rate (Omacini et al. 2006). The root mycorrhizal fungi were not examined In *Dendrobium aqueum*, although the endophytic accumulation was relatively higher in the stems than in the leaf as well as the pseudobulb. The total fungal development rate was also complex on stem segments as related to leaf and pseudobulb parts, which is comparable to *Dendrobium nobile* (Yuan et al. 2009); in *Dendrobium loddigesii*, the leaves contain a greater quantity of endophytic fungi than the stem. This difference might occur because of the sterilant ($HgCl_2$) used; subsequently, the degree of external sterilization was described to greatly overturn repossession by the endophytic fungi (Hyde and Soytong 2008).

17.2.4 Endophytic Microbial Mechanism Responsible for Induced Product Development

Recent investigations showed that when *Dendrobium nobile* was inoculated with *Mycena* sp., and the isolated fungal mycorrhiza from the *Dendrobium officinale* roots sampled, its entire alkaloid percentage was significantly amplified (18.3%), suggesting that mycorrhizal fungi might encourage the biosynthesis of dendrobine. However, the association between host plant and the mycorrhizal fungus is still not very clear, which delays the application of mycorrhizal fungi. Therefore, considering the regulatory machinery of fungal mycorrhiza and refining the dendrobine percentage of *Dendrobium nobile* are of considerable importance.

HMGS and HMGR have been well recognized as equally vital key enzymes in the MVA pathway. Many investigations provided signs of a positive connection between sesquiterpene production and the expression of these two genes. Unexpectedly, this report does not indicate a specific relationship between these two genes and the dendrobine content, as shown by RNA-seq and qRT-PCR. These results suggested that the genes might not have an active function in the process of dendrobine biosynthesis infected by MF23 (Li et al. 2017a, b, c).

In the MVA pathway, the first enzyme, AACT, shrinks two molecules of acetyl-CoA to produce acetoacetyl-CoA in terpenoid backbone biosynthesis. PMK and MVD are crucial ATP-dependent enzymes in the MVA pathway that directly affect isopentenyl diphosphate (IPP) biosynthesis, the building block of sesquiterpene skeletons. The three genes that encode the corresponding enzymes, AACT, PMK, and MVD, were highly expressed with increased dendrobine content. Previous reports confirmed that product accumulation of downstream reactions in the MVA pathway was enhanced in AACT-overexpressing transgenic plants. Therefore, AACT might take a similar role in the dendrobine biosynthesis induced by MF23. In addition, the increases in PMK and MVD expression levels in the model group improved the dendrobine content, which might be the result of increased IPP biosynthesis. In recent years, homologous genes of PMK and MVD have been cloned from *Amomum villlosum*, *Ginkgo biloba*, and *Eleutherococcus senticosus*. One report proved that PMK was highly expressed in the roots of *Aconitum heterophyllum* Wall where the aconite terpenoid alkaloids were synthesized and accumulated. Our results were similar to those in these reports just mentioned, but our work investigated the PMK expression level in *D. nobile* for the first time and was the first report on the effect of the MVD gene on regulating dendrobine biosynthesis, which provided a foundation for further research on MVD and PMK (Li et al. 2017a, b, c).

Sesquiterpene synthases accelerate the development of the sesquiterpene backbone from FPP53. Therefore, these enzymes are widely observed as the rate-determining regulatory phase in MVA pathways. In this study, the expression level of TPS21, a gene encoding sesquiterpene synthase, was negatively correlated with dendrobine biosynthesis. According to the structural features of its catalysate humulene, TPS21 was not the specific sesquiterpene synthase involved in dendrobine biosynthesis. Moreover, diverse reports have proved that different types of sesquiterpene synthases may exist in one plant, and these enzymes compete as they consume the common precursor, FPP, to synthesize different types of sesquiterpenes. Therefore, the low TPS21 expression level might cause more FPP to flux to the biosynthetic pathway of dendrobine, and TPS21 might affect dendrobine biosynthesis irregularly after plants are diseased by MF23. Post-modification enzymes, for example, cytochrome P450, *N*-methyltransferase, and aminotransferase, were important for all secondary metabolic plant pathways, together with alkaloid biosynthesis, hormones, signaling molecules, UV protectants, fatty acids, defense compounds, and pigments. It has been established that about one-half of the projected 19 enzymes in the taxol biosynthesis pathway were determined to be cytochrome P45059. Different studies revealed that by silencing genes encoding aminotransferases, the biosynthesis of relevant alkaloids in the opium poppy and *Camellia simensis* could be specifically concerned (Li et al. 2017a, b, c).

17.3 Application of Bacterial and Fungal Endophytes

17.3.1 Development of the Protocorm of Dendrobium

Dendrobium is among the very important ornamental orchids and is difficult to grow quickly by vegetative means. Propagation is usually performed by seed in vitro, but the difficulty is that this does not produce an unvarying crop and flower color is not stable. The best results are obtained with the development of the protocorm. According to Restanto et al. (2016), using 20 g protocorm in a bioreactor for 8 weeks can produce 18,000 protocorms proficiently with 18,000 uniform orchid seedlings (Restanto et al. 2016).

Since the fungal endophytes that can produce taxol were described by Strobel and Daisy (2003), it was concluded that the appropriate endophytic fungi to ferment and synthesize wide-ranging active ingredients is one active way to resolve the resource deficiency of the few plant derivative compounds. Endophytic fungi, microbes that exist in host plant tissues and cause no deceptive destruction to the host plant throughout the observed phase in their growth cycle, are recognized to yield some intermittent and novel natural mediators with prominent pharmacological activities such as antitumor and antimicrobial (Wu et al. 2015).

An elicitor is a precise factor that can activate enzyme sites during plant biological metabolism, and elicitor stimulation can improve the metabolite content or even produce novel materials (Wang et al. 1999). Elicitors are categorized into two categories dependent on their origin, whether abiotic or biotic. Biotic elicitors have a natural origin and contain polysaccharides, glycoproteins, proteins, and cell wall fragments from bacteria, fungi, and plants. Abiotic elicitors have a nonbiological origin and include metal ions, UV light, and chemically defined compounds. Eliciting cultures with biochemical substances has been the most consistent method and a useful biotechnological means for assessing metabolite production in increased amounts (Radman et al. 2003). Some investigations have also used biochemical elicitors to encourage the production of bioactive substances (Sahu et al. 2013; Largia et al. 2015). By means of a volatile methyl ester of the herb hormone jasmonic acid, methyl jasmonate (MeJA) has been identified as a signaling molecule under biotic and abiotic stress. Some salicylic acid (SA) that caused an alternative pressure on signaling particles was lengthily investigated for its role in prompting plant resistance to pathogens (Rao et al. 2000). Up to the present time, however, MeJA or SA is an active elicitor utilized in various herb cultures (Sivanandhan et al. 2013; Largia et al. 2015; Wang et al. 2016).

17.3.2 The Production of Antibacterial Substances by Endophytes

The secondary metabolites formed by fungal endophytes are greater than those of other endophytic microbe categories, which might show moderate significance in the high occurrence of endophytic fungi isolation from plants (Zhang et al. 2006). With similar purpose, some fungal species appear to have advanced occurrence of isolation and consequently a relatively better means of determining an antibacterial compound formed by related species. The detection of an antibacterial consequence of a crude product of the broth culture or the mycelium is the primary step needed for the encounter of an original antibiotic. It often occurs that the separate substances, including some crude materials, do not have effective antibacterial action themselves but performance synergistically when combined. The identification and structure elucidation of the strongest metabolite is vital in the expansion of an original antibiotic that could possibly be used in therapy.

Two new 10-oxo-10-*H*-phenaleno [1,2,3-de]-chromene-2-carboxylic acids, xanalteric acids I and II, and 11 known secondary metabolites were found from endophytic *Alternaria* sp. extracts, purified from the mangrove *Sonneratia alba* plant found in parts of China (Kjer et al. 2009). The two new substances, xanalteric acids I and II, exhibited feeble antibacterial activity against *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) of 250 and 125 g/ml, respectively. Altenusin showed comprehensive antimicrobial action against numerous impervious pathogens with MICs of 31.25–125 g/ml.

In a recent investigation of antimicrobial action of crude fungal extracts from endophytic fungi (from mangrove), Buatong et al. (2011) analyzed a total of 385 fungal extracts from 150 fungal endophytes by means of an antimicrobial screening method (a colorimetric microdilution procedure). They isolated fungal endophytes from leaves and branches of 12 mangrove species (*Avicennia alba*, *Aegiceras corniculatum*, *Avicennia officinalis*, *Bruguiera parviflora*, *Bruguiera gymnorrhiza*, *Lumnitzera littorea*, *Rhizophora mucronata*, *Rhizophora apiculata*, *Sonneratia caseolaris*, *Xylocarpus granatum*, *Scyphiphora hydrophyllacea*, and *Xylocarpus moluccensis*) screened from mangrove regions in Thailand in the south of Satun, Surat Thani, Songkhla, and Trang states. Ethyl acetate crude extracts were formed from ethyl acetate, culture broth, and hexane extract from the mycelia of fungi, with minimal bactericidal (MB) and minimal inhibitory (MI) concentrations against human pathogens. Ninety-two microbial isolates formed inhibitory substances. Most of the crude extracts (28–32%) repressed *Staphylococcus aureus* growth. Only 2 crude extracts repressed *Pseudomonas aeruginosa* growth, and none of the other extracts repressed *Escherichia coli*. The maximally active fungal extracts were purified from six distinct genera, including *Diaporthe*, *Acremonium*, *Hypoxylon*, *Pestalotiopsis*, *Phomopsis*, *Phomopsis*, and *Xylaria* recovered from *Rhizophora apiculate*, which presented the widest antimicrobial range with low MIC values of 8–32 g/ml against gram-positive bacteria.

Chromatographic extracts separated from cultures grew well in solid or liquid on rice media for the endophytic fungi *Ampelomyces* sp. recovered from the *Urospermum picroides* medicinal plant, which produced 14 natural compounds that were purified based on their ^{13}C- and ^{1}H-NMR as well as mass spectra and evaluation with earlier available data. 3-*O*-Methylalaternin, found from the *Ampelomyces* sp. extracts developed in liquid medium, and altersolanol A, from the fungi developed on solid rice media, showed antimicrobial activity against of the pathogens. 3-*O*-Methylalaternin showed activity with a MIC of 12.5 g/ml against *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *S. aureus*. Altersolanol A had an MIC of 12.5 g/ml against *S. epidermidis* and *E. faecalis*, and 25 g/ml against *S. aureus* (Yu et al. 2010). An analysis by Yagi et al. (1993) had previously found that altersolanol A prevents the growth of bacteria, especially gram positive, and *Pseudomonas aeruginosa* IFO 3080 when verified by means of the broth medium dilution technique (Radic and Strukelj 2012).

Aspergillus sp. was recovered from *Garcinia scortechinii* root samples, a small tree widespread in Malaysia that is frequently used by the native people for postpartum care and peptic ulcers (Ramasamy et al. 2010). Xanthones recovered from the plant host *G. scortechinii* had been found beforehand to prevent methicillin-resistant *Staphylococcus aureus* (Jain et al. 2019). The antimicrobial action of the crude extract of an ethyl acetate agar culture was tested against *Escherichia coli*, *Bacillus subtilis*, and *S. aureus* as well as *Micrococcus luteus* using a disc diffusion process (Radic and Strukelj 2012; Waghunde et al. 2017).

17.4 Endophytic Fungi as Producers of the Same Metabolites as the Host Plant

Some endophytic microbes can produce intermittent and vital bioactive compounds with properties similar to the distinctive properties of the herb host (Wu et al. 2007). This capability is of great importance in that it offers an alternative approach for the need to increase the yield of sluggishly growing and perhaps rare plants and also benefits preserving the world's always diminishing biodiversity. Furthermore, the higher production value of the phytochemical by manipulating microbial growth is easier and more cost-effective, leading to increased obtainability and abridged product marketplace prices (Waghunde et al. 2017). One of the greatest possibilities is the taxol-producing fungal endophytes, but specimen compounds of endophytic fungi with antibacterial action originally occurred in host plants have also been observed (Radic and Strukelj 2012).

Kusari et al. recovered the endophytic fungus identified as *Thielavia subthermophila* from the host plant *Hypericum perforatum* to examine the formation of hypericin, a naphthodianthrone consequential, and its precursor (Kusari et al. 2014). Both compounds confirmed antimicrobial action against several fungi and bacteria, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella enterica*. *Eucommia ulmoides* Oliver is an outdated medicinal plant species widely used in China, and it is one of the chief rich sources of chlorogenic acid. This common medicinal source is in very limited supply currently because of the overexploitation of the native plant, which is now endangered in China. Chen et al. (2010) thought that by recovering a fungal endophyte from *Eucommia ulmoides* which would be suitable to produce a secondary metabolite similar to its host plant they could defend the host plant from extermination and find another way to yield its active ingredients to gratify the need. Chen et al. investigated recovering a fungal endophyte from *Eucommia ulmoides* Oliver and explored whether any of these fungal strains can produce chlorogenic acid (Chen et al. 2010).

Twenty-nine fungal endophytes were recovered on the basis of phenotypic character and were categorized into six major groups: N, B, S, C, A, E. From the antimicrobial action of chlorogenic acid, the recovered endophytic fungi were differentiated as probable makers of chlorogenic acid based on the antibacterial action of their extract. Most showed positive results for antibacterial action and were thus exam-

Table 17.3 Endophytic fungi producing metabolites with antibacterial activity

Endophytic fungi	Plant	Metabolite	Effective against	Method used	Reference
Fusarium solani	*Dendrobium loddigesii* Rolfe; stem, leaf, root	Ethyl acetate extract	*E. coli, Bacillus subtilis, Staphylococcus aureus*	Paper disc diffusion test	Chen et al. (2010)
Fusarium spp., *Phoma* spp., *Epicoccum nigrum*	*Dendrobium devonianum* Paxton; stem, root, *Dendrobium thyrsiflorum*; stem, root	Ethanol extract	*B. subtilis, S. aureus, E. coli*	Agar diffusion test	Xing et al. (2011)
Fusarium nivale	*Dendrobium crumenatum* Sw.	Secondary metabolites	*Candida albicans, C. tropicalis*	Kirby-Bauer disc test	Mangunwardoyo et al. (2011)

ined by high pressure liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), and liquid chromatography (LC)–MS. Chromatographic study indicated that strain B5 might be able to produce chlorogenic acid, even though the yield was comparatively low and was not quite appropriate for a corporate manufacturing level (Table 17.3) (Radic and Strukelj 2012).

17.5 Molecular Approach for *Dendrobium*-related Microbial Endophytes

The rapid growth in microbial molecular biology recently has not permitted outdated microbial examination approaches to be accompanied by the collection of unprecedented amounts of 16S rDNA information (Caporaso et al. 2012). The diverse procedures of molecular approaches, for instance, ribosomal DNA sequencing, single-strand conformation polymorphism (SSCP), amplified ribosomal DNA restriction analysis (ARDRA), random amplified polymorphic DNA (RAPD), terminal restriction fragment length polymorphism (TRFLP), denaturing/temperature gradient gel electrophoresis (D/T-GGE), and novel barcoded amplicon pyrosequencing, are progressively prevalent in the studies of communities of microbes (Bakke et al. 2011). These methods are mostly reliant on 16S rDNA as well as suitable for the examination of microbial samples. Examination of the diversity of endophytic bacteria and their interaction with their host plant is still an identity important for the frontier of microbiology. As per the endosymbiosis concept, the rDNA of chloroplasts and mitochondria demonstrated a higher identity with bacterial rDNA (Rastogi et al. 2013).

Yu et al. (2013) described the universal bacterial primers likely to amplify segment of the chloroplast as well as mitochondrion 16S/18S rDNA, excluding that of endophytic rDNA from *D. officinale*, because the preponderant plant genome is contained in the total genome DNA (Awasthi et al. 2020). It is hence unsuitable for known molecular microbial techniques, including SSCP, ARDRA, RAPD, T-RFLP, pyrosequencing, and DGGE/TGGE, all of which are based on the bacterial 16S rDNAs (Table 17.4).

17.5.1 Identification Approaches

Morphological identification of fungal endophytes is not as expensive as hitherto. For morphological or phenotypic characters, fungal identification, color of mycelia, number of nuclei/young hyphal vegetative cells, and the teleomorph morphology can be intact for species differentiation. However, it is typically hard to recognize orchid endophytic fungi at the species level and sometimes at the genus level because they will not sporulate readily in cultures or unless acquired for at least a few weeks (3–4 weeks) to months or years. Some fungi are still unidentified up to the present (Agustini et al. 2016).

Consequently, internal transcribed spacer (ITS) region molecular sequencing is generally chosen to identify and characterize fungal endophytes, whether alone or included in phenotypic identification. Henceforth, to preserve any wild orchid inhabitants and to reinstate the ex situ preserved plants to a natural habitat, data on their definite 76 fungal associates are vital (Parthibhan et al. 2017).

The molecular technique using DNA sequences was used to confirm the *Rhizoctonia* that was isolated. First, we used ITS rDNA sequences for the analysis, with results closely related to *Ceratobasidium* sp. (JX913817), which belongs to Basidiomycota, with 90–91% similarity. The 28S recombinant DNA sequences were analyzed at that time to confirm the identification of these orchid mycorrhizal fungi because of the low sequence similarity of ITS (Agustini et al. 2016).

17.5.2 Construction of Specific Primers

Many specific primers have been considered to distinguish bacterial and fungal endophytes in some plants. A primer 799f (*E. coli* numbering, 5′-AACMGGATTAGATACCCKG-3′) was considered that can increase maximum bacterial sequences with the elimination of chloroplast DNA (Yu et al. 2013).

17.6 Environmental Abiotic Factors Affecting *Dendrobium* Plant Products

Alkaloids are a type of secondary metabolite of plants with significant roles in defensive responses to ecological pressures. For instance, binary trauma induced an upsurge in indole alkaloid biological synthesis in the plant *Catharanthus roseus*.

Table 17.4 Specific primers, sequences, and tools used for the molecular identification of endophytes

Primer	Sequence	Software	Plant name	Microbes	Reference
Primer pairs: ITS1, ITS4	ITS1: (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)	BLAST, Clustal X	*Dendrobium nobile*	Fungi	Yuan et al. (2009)
Primer pairs: fC3/rM6-1, fC3/rM6-2, fC3/rM6-3, fC3/rM7, fC4/rM6-2, fC4/rM7, and fC4/rM6-3	5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3′	BLASTN Algorithm, MEGA4.0	*Dendrobium officinale*	Bacteria	Yu et al. (2013)
Primer pairs: NL1/NL4 and ITS1/ITS4	5′-GCATATCAATAAGCGGAGGAAAAG-3′/ 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and 5′-CTTGGTCATTTAGAGGAAGTAA-3′/ 5′-TCCTCCGCTTATTGATATGC-3′	Sequence Alignment Editor (Se-Al) v.2.0, CLUSTAL X v.1.8	*Lauraceae* and *Rutaceae*	Fungi	Ho et al. (2012)
Primer pairs: ITS1 and ITS4	5′-TCC GTA GGT GAA CCT GCG G-3′ and 5′-TCC TCC GCT TAT TGA TAT GC-3′	BLAST, Clustal X 2.1	*Dendrobium officinale*	Fungi	Jin et al. (2017)

Under deficiency conditions, the alkaloid quantity in the motherwort roots was improved to 1.7 fold more developed than the control sets, with a substantial change ($p < 0.01$). When infected by *Ceratocystis fimbriata*, several alkaloids in mango increased at different degrees of resistance to infection. Similarly, increased levels of MF23, as a foreign invader for *D. nobile*, might elicit plant defense, which is likely to include the alkaloid (dendrobine) biosynthetic pathway (Li et al. 2017a, b, c).

17.7 Conclusion and Future Prospects

Dendrobium plants have been widely used therapeutically as medicinal herbs and in nutraceutical production since early times in China. The previous three decades have seen growing study of the polysaccharides and their alkaloids isolated from *Dendrobium* herbs. Bioactivity including antitumor, immunomodulatory, antidiabetic, and antioxidant characteristics has been observed to originate from *Dendrobium* products. There are numerous important features of orchids that require immediate consideration and which will only be conceivable with microbial transformation. The major challenge for such investigations, however, is to produce large populations of orchids for assessment and analysis, as orchid plants are overall very sluggish and exclusive in growth. The identification and metabolic products of endophytic microbial species present extreme challenges for any plant pathologist. Endophytic microbial taxonomy has been continually varying for decades and continues to change, so assigning a name to a particular microbial species can be equally problematic as well as divisive.

It is significant to notice that producing the antibacterial constituents of these fungi has been accomplished in a test center, and more effort is required to isolate as well as purify these fungi with the aim to determine their structures and mechanisms. Molecular analysis is rapidly becoming the norm for classification and identification of endophytic microbial species because it offers the most reliable and consistent method for recognition of alterations between different groups. Pathogenicity tests can then be conducted to further characterize a species and its disease-causing capabilities. Once proof of identity and pathogenicity are established, separation procedures can be applied as needed to avoid the progress of disease into new areas. Therefore, further comparative investigations of chemical constituents among *Dendrobium* plants and its endophytic fungi are certainly desirable.

Because endophytic fungi are sought for in plants that have established use in traditional medicine or which grow in areas of great biodiversity, it is understandable that the discovery of many possibly useful metabolites can be expected. Concentrating on the examination of endophytic fungal range, relationships among endophytic fungi and their host herbs, looking for natural bioactive compounds thus formed, and refining the productivity of many potential natural substances, we can take primary advantage of genetic and molecular technologies and microbial fermentation developments for the detection of much-needed antibiotics to control contagions caused by multidrug-resistant microbes. It is very clear that we have a similarly long path to accomplish the ultimate goal.

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