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Research Article

A Review of Potential Applications of Orchid Endophytes

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Abstract

With the increasing use of microflora, including intestinal bacteria, in medical and health care, research is also expanding to plant microflora. Plant tissues, similar to those of animals, harbor resident microorganisms. Some microorganisms living symbiotically in plant roots are believed to attract other fungi and bacteria, forming a microbiome that promotes plant growth. A group of microorganisms act as a core of the microbiome, collectively referred to as endophytes. Endophytic microbes, especially fungi and bacteria assist plants by supplementing nutrient acquisition, synthesizing plant growth regulators and provide immunity to plants against phytopathogens, biotic and abiotic stresses. Orchids, belonging to the diverse family Orchidaceae, are renowned for their vibrant and fragrant blossoms and thrive in nearly all terrestrial ecosystems except the poles and extremely dry deserts, with their greatest diversity found in the tropical region. Orchids are economically important for their ornamental value, medicinal properties, and use in food products. Their seeds lack endosperm, and they must depend on endophytes from the very early stage of life: germination, growth, and adaptation. Recent research into orchids has focused on isolation and identification of the endophytes that directly or indirectly contribute to the growth and development of orchids as well as the production of valuable secondary metabolites. This review considers both the role such endophytes play and explains how such symbiotic partners can be used in the plant tissue culture technique to help increase plant growth, productivity, protection against pathogens and stress resistance.

Keywords: Orchidaceae; Orchid Endophytes; Microflora

Introduction

The Orchid family, Orchidaceae is the diverse, widespread and second biggest group of flowering monocotyledonous plants, which blooms with colorfulness and fragrance. Orchidaceae includes approximately 28,000 recognized species spread across 763 genera [1]. Orchids are used in traditional medicine to treat various ailments. Additionally, orchids are ingredients in food and flavoring products, such as the tubers of Vanilla orchids in international trade. These are one of the most significant cosmopolitan plants family that have ecologically adapted to almost every habitat except Antarctica and dessert. Most orchids are epiphytes which grow anchored to trees or shrubs. Others are terrestrial and lithophytes. Epiphytic orchids are mostly habitant to tropical and subtropical environments, whereas terrestrial orchids occur in all climatic zones. The epiphytic species have peculiar valamenoid roots.

Orchids possess the smallest seeds among all flowering plants. An average orchid seed is about the size of a dust particle [2]. Orchids count on quantity before quality in reproduction strategy. Their reproductive strategy prioritizes quantity over quality. Instead of generating a few nutrient-rich seeds, orchids produce millions of seeds per pod, which are easily dispersed by wind over vast areas. Once ripe, these seeds scatter like dust. Most orchid species lack endosperm, and only a small fraction of the seeds ever germinate [3]. Symbiotic relationships with various mycorrhizal fungi that provide them with necessary nutrients are important for germination. So, almost all orchid species are mycoheterotropic during germination and rely on fungi symbiosis to complete their lifecycle [4]. Some orchids can join up with many different species of fungi whilst others only accept a very specific fungus to enter their roots. A few orchids don't need any fungus at all for their germination. *Disa uniflora*, for example, is the unique name for South Africa's most famous orchid is a remarkable exception among terrestrial orchids. So, why are orchids a big deal? In short, because they are incredibly difficult to propagate from seed. It is extremely difficult to propagate rare orchids without environmental mycorrhizal fungi. Orchids must be grown on special nutrients media in the laboratories to be propagated easily. This is the main reason why the Earth is not rich in orchids and orchids are endangered species [5].

Even though orchids might be considered the pinnacle of plant evolution, they are among the most threatened flowering plants. Overzealous collection and habitat loss have pushed many species towards extinction in the wild. Due to the threat from overcollection, all orchids have been placed in the Appendix II of Convention on International Trade in Endangered Species (CITES) [6]. Orchids hold the highest value in commercial horticulture, making them susceptible to illegal poaching. For instance, *Paphiopedilum canhii*, first discovered in the mid-2009 and 99.5% of its population was extinguished in only six months of exploitation. Orchids are also collected for ethnobotanical purposes. In the Chinese medicinal trade, pseudobulbs of *Dendrobium* species are used to heal swelling of hands and legs, while tuberous terrestrial orchids are collected in East sub-Saharan Africa to produce a cake called chikanda. Similarly, in Turkey, tubers of terrestrial orchids are used to make an extract known as salep, which is used in ice cream production. Due to their complex ecological interactions with pollinators, mycorrhizal fungi, and other plants and animals, orchids are often the first biological indicators of ecosystem decay [7]. Restoring ecological stability conducive to orchid persistence may take many decades, given their reliance on insect pollinators and mycorrhiza.

Orchidaceae is the largest family of flowering plants in Nepal, comprising 108 genera and 501 species including 20 endemic orchids [8]. They are highly popular for their unique shape, size, and visual appeal, featuring colorful flowers, and variously shaped pseudobulbs. The diversity in size, shape, and color of their flowers makes them highly sought after for horticultural purposes (Figure 1). Most of the wild orchids in Nepal are under threat, and some are at the verge of extinction that require urgent conservation efforts. *Dactylorhiza hatagirea,* commonly known as *paanch aunle*, is endemic to the Himalayas and is named for its resemblance to a palm. Its collection, use, and sale are strictly prohibited. Another vulnerable species is *Gastrodia elata*, which risks extinction if government export licenses continue to be issued for its trade. *Paphiopedilum venustum,* also known as the Venus slipper, is classified as endangered by the IUCN. Similarly, the Lady Slipper Orchid, recognized for its distinctive shoe-shaped flowers, faces significant threats due to overharvesting [9]. These species highlight the urgent need for sustainable management and conservation policies.

Each plant hosts a unique microbiome, and orchids are among the most significant plants in this regard. The history of orchids is deeply intertwined with human civilization, dating back approximately 4,000 years in China and Japan, where they were initially used for medicinal purposes. Orchids were employed as remedies for various ailments, including coughs, fevers, wounds, and infections [10]. New orchid species continue to be discovered. Scientists from Madagascar, the U.S., and Europe have recently identified a new orchid species discovered in the forest canopies of central Madagascar. The orchid, named *Solenangis impraedicta*, features a nectar spur measuring 33 centimeters in length, the longest known relative to its flower size. Additionally, a newly identified orchid species in Japan, named *Spiranthes hachijoensis*, has petals that appear as if they are spun from glass [11]. With the discovery of new orchid species, there is also potential for uncovering new endophytes associated with them.

Importance of the study

Orchids are one of the richest sources of endophytic microorganisms. Studying these endophytic microorganisms in orchids holds immense importance for both ecological and practical reasons. Although orchids are ecologically specialized and diverse plants, its survival, growth and development immensely depend on its symbiotic associations with microorganisms, particularly endophytes. These endophytes not only play crucial role in seed germination, production of plant growth promoting substances, nutrient acquisition but also enhances resistance to both biotic and abiotic

Figure 1: (a ,b) Epiphytic orchids *Coelogyne nitida* and *Coelogyne cristata* found in the Phulchowki forest in Nepal (c-e) Various Cymbidium orchids for sale at Floriculture Development Centre, Nepal.

stresses. Orchids are also one of the difficult plants to propagate because orchid seeds cannot germinate without endophytic microorganisms in the wild. It has contributed to the rarity of various orchid species in nature. Understanding the orchid-endophytic microbiome interaction is vital for its conservation. Furthermore, it has broader implications in agriculture, biotechnology and environmental sustainability [12]. Various orchid associated endophytes produce bioactive compounds with potential applications in agriculture as biocontrol agents and fertilizers, in plant tissue culture as phytohormones, and in pharmaceuticals as antimicrobial and antioxidant substances. Likewise, in order to study plant-microbe interactions in nutrient poor and symbiosis dependent environments orchids can serve as model system.

Hence, studying orchid microflora is crucial for several reasons. It plays a vital role in orchid conservation by enhancing our understanding of the ecological relationships between orchids and microorganisms, which can help develop effective propagation and preservation strategies. Microorganisms such as bacteria and fungi assist orchids in nutrient acquisition, growth, and stress tolerance, significantly impacting their survival and resilience. Additionally, orchid-associated bacteria produce bioactive compounds with potential applications in sustainable agriculture and other sectors. The symbiotic associations orchids form with microorganisms, whether mutually beneficial or exploitative, are integral to maintaining biodiversity and ecosystem balance. Furthermore, orchids are often regarded as environmental health indicators, reflecting the overall well-being of their ecosystems [13]. This study aims to expand the scientific basis for selecting orchids as the source of endophytic microbes with potential benefits in ecological conservation, horticulture, and commercial propagation techniques of orchids.

Role of endophytic microorganisms in orchids

Microbes are essential for sustaining life on Earth. The plant microbiome is crucial for plant health and productivity, drawing significant attention in recent years. An example of the importance of plant-microbe interactions is the role of mycorrhizal fungi. Molecular evidence suggests that their associations with green algae were critical to the evolution of land plants around 700 million years ago [14]. Plant-associated microbes also play vital roles in global biogeochemical cycles.

Plant tissues, similar to those of animals, host resident microorganisms mainly bacteria and fungi. Among these are endophytes, a group of microorganisms that act as a core, attracting other resident bacteria and forming a microbiome. This "core" concept emerged from data showing that endophytes are centrally located within many microbiomes when the microbial networks around plant roots are visualized. Although only a few endophytes have been identified so far, it is estimated that over 10,000 different microorganisms function as endophytes [15].

An endophyte which acts as an endosymbiont (often bacteria, fungus or actinomycetes) lives within a plant without causing apparent disease significant morphological changes. Some endophytes enhance host growth, nutrient acquisition or improve stress tolerance of plants. Recent research on the isolation and identification of endophytes from host plants can build our understanding about how plants adapt to their native environments. Endophytes can reside in diverse plant parts, such as buds, seeds, stems, roots, leaves, flowers, fruits, and even in dead or hollow hyaline cells [16]. These microorganisms, which may be bacteria (such as actinomycetes or mycoplasma) or fungi, establish themselves within plant tissues. Over 200 bacterial genera spanning 16 phyla have been identified as endophytes, with the majority belonging to the phyla Actinobacteria, Proteobacteria, and Firmicutes. The estimated number of endophytic fungi is around one million, based on the 6:1 ratio of fungal to plant species. However, this figure could be significantly higher, as only about 5% of fungal species have been described so far. Endophytic fungi are broadly categorized into two groups based on their phylogeny and life history. The first group, clavicipitaceous endophytes, primarily infect grasses in cooler regions. The second group, non-clavicipitaceous endophytes, are found in asymptomatic tissues of non-vascular plants, ferns, conifers, and angiosperms, typically from the Ascomycota or Basidiomycota groups [17].

In orchids, the study of microbial endophytes has been mainly focused on mycorrhizal fungi because of their important role in orchid seed germination and early plant development. Mycorrhizal fungi colonize the roots of both terrestrial and epiphytic orchids, where they form intracellular hyphal coils named 'pelotons' [18]. Basidiomycetes in the form genus Rhizoctonia are commonly found in the roots of photosynthetic orchid species. Whereas ascomycetes and basidiomycetes are able to form ectomycorrhizal symbioses with neighboring plants usually colonize the roots of non-photosynthetic or partially photosynthetic orchid species. Various studies have shown the role of mycorrhizal interactions in orchids in plant growth promotion. By contrast, the diversity and role of endophytic bacteria have not been extensively explored in orchids. Although bacterial endophytes have been identified in commercially valuable epiphytic orchids, such as *Dendrobium* ,*Vanilla* and *Cymbidium* species and other hybrids [19].

Orchid seeds typically lack endosperm and depend on mycorrhizal fungi for nutrient uptake during germination. Endophytes supply vital nutrients such as carbon, nitrogen, and phosphorus, supporting seed germination and initial plant development. In some studies, the role of endophytic microorganisms in promoting orchid seed germination and plant growth was demonstrated in vitro. This further indicates their potential plant growth promoting activity. Endophytic fungi associated with different parts of orchid are found to secrete various kinds of plant hormones such as auxin, cytokinins, abscisic acid, gibberellic acid and ethylene that are effective in overall growth and development of plants. Furthermore, endophytes play a significant role in the solubilization of inorganic phosphate and ammonia synthesis [20]. Thus, symbiotic association of plants with endophytic microbes may help increase the availability of nutrients which might be helpful for high productivity in biomass of a host species (Figure 2).

Endophytes produce bioactive secondary metabolites, including alkaloids, flavonoids, and terpenoids, which help protect orchids from diseases and pests while enhancing their medicinal properties. Endophytic microorganisms significantly contribute to orchids' stress tolerance by establishing mutualistic relationships. They inhabit the internal tissues of orchids without causing harm, providing various advantages that help the plants withstand both abiotic and biotic stresses. Abiotic stress resistance encompasses challenges such as drought, extreme temperatures, salinity, and heavy metal toxicity, while biotic stress resistance involves defense mechanisms against pathogens and herbivorous insect attacks. Endophytes enhance the resilience of orchids, enabling them to thrive in challenging, nutrient-deficient environments, which is crucial for the preservation of rare and endangered species [21]. Additionally, they improve rhizosphere health by fostering beneficial microbial diversity, indirectly supporting the growth and sustainability of orchid populations.

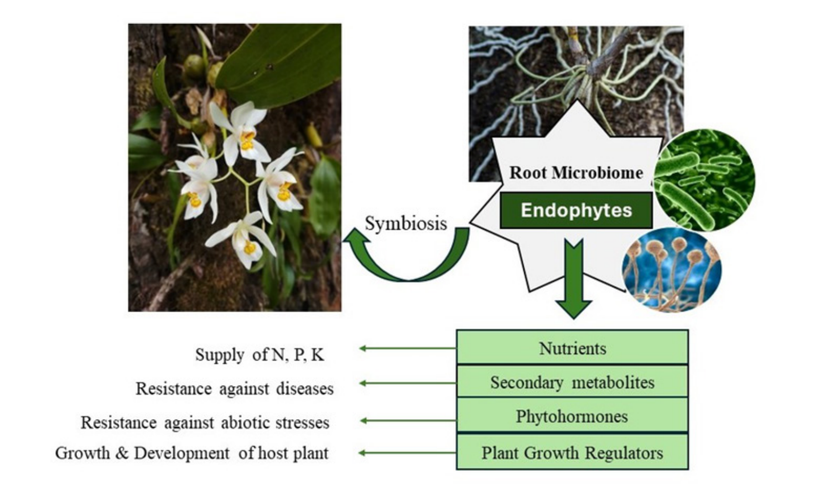

Figure 2: The symbiotic relationship between an orchid plant and root microbiome. Endophytic fungi and bacteria act as a core of the microbiome supplying essential nutrients, promoting growth and development to the host plant and providing resistance against biotic and abiotic stresses.

Production of secondary metabolites by orchid endophytes

Endophytic microorganisms associated with orchids are a prolific source of bioactive secondary metabolites with significant ecological, medicinal, and biotechnological potential. These metabolites, including alkaloids, flavonoids, terpenoids, phenolic compounds, polyketides, and non-ribosomal peptides, play a vital role in orchid adaptation [22]. Alkaloids produced by endophytes exhibit strong antimicrobial and insecticidal properties, protecting orchids from pathogens and pests. Flavonoids and phenolic compounds act as powerful antioxidants, enhancing stress tolerance by mitigating oxidative damage and providing UV protection. Terpenoids, known for their antimicrobial, anti-inflammatory, and anticancer properties, contribute to the plant's defense and potential pharmaceutical applications. Additionally, polyketides and non-ribosomal peptides produced by endophytic fungi are recognized for their potent antimicrobial and anticancer activities.

Natural vanilla flavor is extracted from the orchids, *Vanilla planifolia*. Many compounds have been isolated and identified from vanilla beans that give the signature vanilla taste. Four major flavor giving compounds are p-hydrooxybenzoic acid, p-hydroxybenzaldehyde, vanillic acid and vanillin [23]. Like other orchid species, many endophtcis fungi are found to be associated with vanilla pods and plants such as *Ceratobasidium spp*, *Fusarium proliferatum*, *Pestalotiopsis microspore*, *Thanatephorus spp* and *Tulasnella spp*. Fungal isolates were found to convert vanilla flavor related metabolites in vitro in green vanilla pods. *Pestalotiopsis microspore* caused the formation of additional vanillin and increased vanillyl alcohol levels in green vanilla pods [24]. This study makes it clear that endophytes play a major role in the quality of cured vanilla beans. *Cymbidium aloifolium* is a potent medicinal epiphytic orchid in the Indian system of medicine, traditionally used in treatment of many human ailments. In a study, 165 endophytic fungi were isolated from root, leaf, and stem of of *C. aloifolium*. *Aspergillus terreus, Colletotrichum gleosporidioides, Penicillium chrysogenum* and *Alternaria alternata* are the major endophytic fungi isolated from various parts of the orchid. These endophytic fungi produced different extracellular enzymes like amylase, lipase, laccase, protease, pectinase, cellulase and phosphatase. *Colletotrichum gleosporidioides* isolated from leaf produced significant phosphatase followed by *Alternaria alternata* isolated from flower [25]. These are promising sources of industrially useful enzymes.

These secondary metabolites not only safeguard orchids from biotic and abiotic stresses but also strengthen their symbiotic relationships by improving nutrient exchange and overall resilience. Beyond their ecological significance, secondary metabolites from orchid endophytes offer significant potential for pharmaceutical, agricultural, and industrial uses [26]. They are being investigated for the creation of novel antibiotics, anticancer therapies, and biofertilizers, benefiting both plant health and human well-being. Their diverse chemical profiles make them a valuable resource for discovering new bioactive compounds to tackle pressing issues like antibiotic resistance and sustainable agricultural practices.

Role of orchid endophytes in growth and development of plants

Plants, similar to humans, require essential elements such as air, light, water, and nutrients to maintain their health and vitality. Deficiencies in any of these critical factors can impair their growth, potentially leading to plant mortality. To support optimal development, plants rely on several key nutrients, with nitrogen, phosphorus, and potassium being the most crucial. Nitrogen plays a pivotal role in promoting vegetative growth, contributing to the plant's lush green foliage. Phosphorus is essential for cell division, flowering, seed production, and the establishment of a strong root system. Potassium enhances the plant's ability to resist diseases and contributes to the development of sturdy stems. The mycorrhizal fungus positively affects P, K, Ca, Mg content in shoots and Zn content in roots, while the endophytic fungus improvs N, P, Ca accumulation in shoots and roots [27]. A study conducted on *Dendrobium cv. Earsakul* explored the effects of nutrients and the endophyte *Piriformospora indica* on orchid growth. Results showed that combining organic and inorganic nutrients with *P. indica* boosted growth, root volume, and nutrient uptake, particularly phosphorus, potassium, and calcium. *P. indica* has been found to enhance plant growth in trifoliate orange by promoting phosphorus uptake through increased soil phosphatase activity and the expression of root phosphate transporter genes [28].

Endophytic association helps in the production of many important plant hormones such as auxin, a hormone which plays an important role in organogenesis, cell expansion, cell division, cell differentiation and gene regulation. Endophytes such as *Bacillus, Streptomyces, Pseudomonas, Erwinia*, *Burkholderia,* and *Nocardia* colonized in *Paphiopedilum* helped produce auxin. Endophytic bacteria *Pseudomonas sp., Flavobacterioum sp* were also shown to play a significant role in auxin production in *Pholidota articulata* [29]. The interaction of these rhizobacteria, especially *Enterobacter* and *Bacillus* species, with seeds and seedlings resulted in high rates of germination and growth and better acclimatization. Orchid endophytes can help plants in other ways, too. Two endophytic bacteria isolated from *Cymbidium eburneum*, *Paenibacillus lemtimorbus* and *Paenibacillus macercus*, have the potential to synthesize auxin [30]. These endophytes were shown to increase the shoot length, root length, and number of plantlets and to promote survival during acclimatization. Similarly, there are many other endophytic bacteria and fungi that help in plant growth as shown in Table 1 and 2.

In a study done in Thailand, 13 endophytic fungi were isolated from three species of orchids *Spathoglottis affinis*, *Paphiopedelum bellatulum* and *Phaius tankervilleae*. Fungal isolates belonging to genus *Tulasnella* and *Colletotrichum* produced high levels of indole-3- acetic acid (IAA) in culture medium. The bioassays with kidney bean (*Phaseolus vulgalis*) cuttings, corn (*Zea mays*) seeds and rice (*Oryza sativa*) coleoptiles were used for estimation of the biological activities of IAA produced by the selected fungal isolates compared with standard IAA. The evaluation of fungal IAA biological activities revealed that all fungal IAA could improve the root formation of kidney bean cuttings, the corn seeds germination and elongation of corn roots and elongation of rice coleoptiles were like the results of treatment with standard IAA, the positive control [31].

Table 1: List of endophytic bacteria isolated from various orchid species and their role in plant growth and protection against pathogens

Orchid Species	Endophytic bacteria	Role	References
Cymbidium eburneum	*Paenibacillus macerans Paenibacillus lemtimorbus*	Auxin production Seedling growth	[31]
Chloraea barbata	*Collimonas pratensis*	Phosphate solubilization Inhibition of fungal growth	[33]
	Dyella marensis Luteibacter rhizovicinus	Siderophore production	
Dendrobium aphyllum	*Tulasnella sp., Trichoderma sp.*	Seed germination	[34]
Paphiopedilum appletonianum	Streptomyces sp., *Bacillus sp., Erwinia sp.*	Indole-3-acetic acid (IAA) production	[30]
Pholidota articulata	Pseudomonas sp., Flavobacterioum sp.	Indole-3-acetic acid (IAA) production	[30]
Spiranthes cernua	*Enterobacter sp.,* Bacillus sp.	Indole-3-acetic acid (IAA) production	[35]
Doritaenopsis Jiuhbao	*Mycobacterium* strain Mya-zh01	Facilitates plant growth and seed germination	[36]
Dendrobium sp.	*Dyella jiangningensis, Bacillus megaterium*	Antimicrobial activity against pathogenic fungi and bacteria	[37]

In another study, growth promoting effects of endophytic fungi of the velamen roots of a wild epiphytic orchid *Acampe praemorsa* on an ornamental orchid, *Dendrobium sp*. was observed. Five endophytic fungal species, *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma atroviride, Endomelanconiopsis endophytica* and *Diaporthe eucalyptorum* were isolated from the velamen roots. *A. praemorsa* were found to produce a significant amount of plant hormone, indole-3-acetic-acid (IAA). The growth-promoting capacity of these fungi was tested on the ornamental orchid. A significant increase in the chlorophyll content in the leaves of treated plants was shown by *Endomelanconiopsis endophytica.* Moreover, *T. asperellum* and *D. eucalyptorum* showed a significant increase in the fresh weight of treated plants. Whereas dry weight of leaves in treated plants were significantly increase by *T. asperellum* and *E. endophytica* [32]. This experiment proved that the endophytic fungal isolates from the wild orchid synthesize bioactive compounds including IAA that can promote growth in ornamental orchids such as *Dendrobium sp*. Hence, the endophytic fungal isolates from wild orchids are proved significant to enhance the production of other ornamental orchids.

Table 2: List of endophytic fungi isolated from various orchid species and their role in plant growth and protection against pathogens.

Orchid Species	Endophytic Fungi	Role	References
Vanilla planifolia	*Fusarium proliferatum*	Increased vanillin production	[25]
	Pestalotiopsis microspora		
Vanda cristata	*Agaricus bisporous Mycolepto discus*	Auxin synthesis phosphate solubilization	[38]
Dendrobium nobile	*Canavalia rosea*	Improves acclimatization	[39]
	Trichoderma chlorosporum	Potential biocontrol and plant-growth promoting capacity	
	Xylaria sp.	litter decomposer once the hosts undergo senescence	
Cymbidium aloifolium	*Aspergillus terreus, Colletotrichum gleosporidioides, Penicillium chrysogenum, Alternaria alternata*	Production of extracellular enzymes like phosphatase, cellulase, amylase, protease, pectinase, lipase, and laccase	[26]
Gastrodia elata	*Mycena dendrobii*	Acceleration in seed germination and enhancement of fresh tuber yield	[40]
Dendrobium loddigesii	*Fusarium sp.*	Promotes host growth, performs activities against plant and clinical pathogens, and has different antibiotic coverage	[41]
	Pyrenochaeta sp.	Improves plant growth and inhibit microbial activity	
Spathoglottis affinis	*Tulasnella sp., Colletotrichum sp.*	Indole-3-acetic acid (IAA) production	[32]

Stress resistance in orchids due to endophytic microbes

Endophytes has been shown to help its host overcome both biotic and abiotic stresses. Biotic stresses include disease causing pathogens present in the environment and abiotic stresses include drought, abnormal pH, salinity, extreme cold and heats, free radicals, and reactive oxygen species [42]. The secondary metabolites from orchids endophytes, including alkaloids, flavonoids, terpenoids, phenolic compounds, polyketides, and non-ribosomal peptides, play a vital role in orchid defense and stress adaptation [43].

Abiotic stress resistance in orchids due to endophytic microbes

Abiotic stress refers to environmental factors that are not biological but negatively influence plant growth, development, and survival. Orchids, being highly sensitive to environmental fluctuations, are especially susceptible to different forms of abiotic stress. These stresses can greatly affect their physiological processes, metabolism, and overall health, particularly in their diverse natural habitats, which range from tropical rainforests to arid areas. Orchids are vulnerable to several types of abiotic stress, each of which impacts their growth and survival. Drought stress, particularly in epiphytic species growing on tree branches, leads to water scarcity, reducing photosynthesis, causing stomatal closure, and hindering nutrient uptake, which ultimately affects growth and flowering. Temperature extremes, both high and low, disrupt metabolic processes; high temperatures induce protein denaturation and oxidative stress, while low temperatures cause cellular damage through ice crystal formation and impaired enzyme activity. In saline environments, orchids suffer from ion toxicity, osmotic stress, and nutrient imbalances, with excessive salt hindering water absorption and nutrient uptake, resulting in stunted growth and chlorosis. Orchids growing in polluted soils or mining areas are exposed to heavy metals like cadmium, lead, and arsenic, which can cause oxidative damage, enzyme inhibition, and disrupt cellular functions. Additionally, light stress can be detrimental, as insufficient or excessive light impairs photosynthesis and causes photoinhibition. Shade-loving orchids may experience leaf burn under intense light, while low light conditions can reduce flowering and biomass accumulation [44].

Endophytic microbes play a vital role in helping orchids cope with various abiotic stresses. Abiotic stress mechanisms primarily involve osmotic adjustment, antioxidant production, nutrient enhancement, and regulation of phytohormones [45]. In response to drought stress, these microbes enhance root growth, improve water uptake, and regulate osmotic balance by producing osmo-protectants like proline and glycine betaine, which maintain cellular integrity under water-deficit conditions. Regarding temperature stress, orchids associated with endophytic fungi exhibit greater tolerance to extreme temperatures, partly due to the production of heat-shock and antifreeze proteins by the microbes, which stabilize cellular structures and protect against thermal damage. In saline environments, endophytes help orchids manage salt stress by promoting ion homeostasis, limiting sodium uptake, and increasing potassium uptake, while also producing exopolysaccharides that protect roots from salt-induced osmotic stress [46]. Additionally, some endophytic microbes can mitigate heavy metal stress by immobilizing heavy metals through biosorption and bioaccumulation, as well as producing enzymes such as phytochelatins and metallothioneins to detoxify these harmful metals.

Orchids generate negative oxygen ions during photosynthesis through processes like tip discharge or the photoelectric effect. These ions play important roles in air sterilization, dust removal, and delaying aging. However, global warming and rising temperatures may reduce orchids' ability to produce these ions. Under high-temperature stress, *Phalaenopsis aphrodite* showed a significant decrease in its capacity to produce negative oxygen ions. The study found that high temperatures caused notable changes in the structure of the root endophytic microbial community. The enrichment of beneficial microbes, such as *Enterobacteriaceae* bacteria and *Rhodotorula* fungi, boosts proline production, stabilizes the endophytic environment in *P. aphrodite* roots, and enhances the plant's heat resistance. The increased microbial abundance also contributes to the production of negative oxygen ions. Additionally, peroxidase levels rose by 102%, and proline content increased by 35% under high-temperature stress [47]. Osmolytes such as proline have the potential to serve as effective signaling molecules for managing heat stress in plants. One approach to creating heat resistant plants could involve engineering them with genes that promote proline synthesis [48]. Alternatively, a more sustainable and cost-effective approach would be the incorporation of proline-producing endophytes into plants, leveraging their natural ability to enhance stress tolerance.

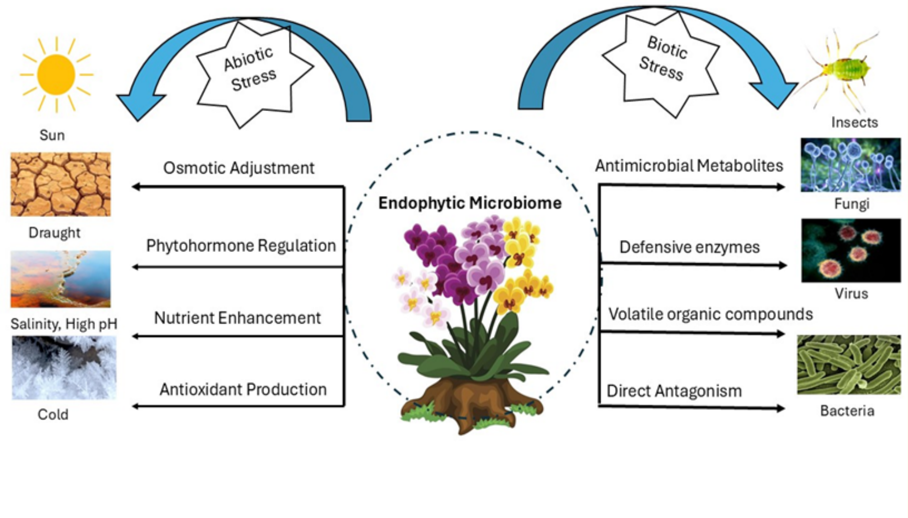

Figure 3: Mechanism of action in endophytic microorganisms against various abiotic and biotic stress.

Biotic stress resistance in orchids due to endophytic microbe

Orchid pests can be broadly categorized into insects, mites, pathogens, and nematodes, all of which can severely affect the growth, flowering, and overall health of orchids. Insects like mealybugs, scale insects, aphids, whiteflies, thrips, and caterpillars damage orchids by feeding on roots, stems, leaves, and flowers. Mealybugs, appearing as cotton-like masses, suck sap and transmit viruses, causing yellowing and stunted growth. Scale insects attach to plant parts, secreting waxy coatings that weaken the plant. Aphids and whiteflies feed on sap, causing leaf yellowing and promoting mold growth. Thrips damage flowers and leaves, causing silvering and streaking while spreading viruses. Caterpillar larvae chew on leaves and flowers, resulting in significant damage. Mites, such as spider mites and cyclamen mites, are common orchid pests. Spider mites feed on leaf undersides, causing stippling and leaf drop, particularly in dry, warm environments, while cyclamen mites attack growing tips, leading to deformed flowers and stunted growth in cooler climates [49]. Pathogens, including fungi like *Fusarium* and *Phytophthora*, cause root and crown rot, while bacteria such as *Pseudomonas* and *Xanthomonas* lead to soft rot and blackened leaves. Viruses like Orchid fleck and Cymbidium mosaic virus cause mottling and distortion. Root-knot nematodes infect roots, disrupting nutrient uptake and causing plant decline. These pests and pathogens threaten orchid health, requiring effective pest management.

Endophytes are increasingly recognized for their ability to protect orchids through direct deterrence of pests and by boosting the plant's own defense mechanisms. Biotic stress mechanisms in orchids involve several strategies, including induced systemic resistance (ISR), antimicrobial metabolite production, volatile organic compounds (VOCs), and direct antagonism [50]. Numerous endophytes have demonstrated potential in managing pest-related issues in orchids by producing bioactive compounds, inducing plant resistance, or attracting natural predators. In terms of pathogen defense, endophytes activate ISR, promoting the production of defense enzymes such as chitinases, glucanases, and peroxidases, as well as secondary metabolites like alkaloids, flavonoids, and terpenoids. Regarding insect herbivory, endophytes contribute to orchid protection by synthesizing toxic secondary metabolites or VOCs that either repel herbivorous pests or attract natural predators, thereby reducing pest-induced damage [51]. These mechanisms collectively enhance the plant's resilience to biotic stress.

Several endophytic species, including *Pyrenochaeta sp.*, *Fusarium sp.*, *Dyella jiangningensis*, and *Bacillus megaterium* (as detailed in Tables 1 and 2), assist various orchid species in combating phytopathogens by exhibiting antimicrobial properties. Additionally, *Pestalotiopsis sp.*, an endophyte isolated from *Dendrobium officinale*, demonstrates both antifungal and antioxidant activities.

This endophyte has been shown to effectively control pathogens such as *Cryptococcus neoformans*, *Candida*, *Trichophyton rubrum*, and *Aspergillus fumigatus* [52]. Dendrobium is one of the largest genera in the Orchidaceae family and is known for its significant clinical effects on human stomach and kidney health, as noted in Chinese pharmacology. Pharmacological studies have reported the beneficial effects of *Dendrobium* in treating liver disease, cancer, hypoglycemia, and gastric ulcers likely due to the presence of bioactive polysaccharides. Endophytic microbes associated with medicinal orchids, like *Dendrobium*, may produce similar bioactive metabolites both in vitro and within host plant tissues [53]. These microbes are also valuable sources of secondary metabolites with antimicrobial activity. In a study utilizing Illumina MiSeq sequencing to analyze the V5–V7 regions of the bacterial 16S rRNA gene, combined with culture-dependent methods, 165 cultivable endophytic bacteria were isolated from *Dendrobium* stem samples. The antimicrobial activity of these isolates was tested using the Kirby-Bauer method. The strains were categorized into 20 genera, with *Curtobacterium* and *Bacillus* being the most dominant [37]. The antimicrobial efficacy of these endophytic bacteria was evaluated against various plant pathogens, including *Athelia rolfsii*, *Myrothecium roridum*, and *Pectobacterium carotovorum subsp.*, which affect a broad range of agriculturally and scientifically significant plants. *Athelia rolfsii* causes collar rot in sunflowers. *Myrothecium roridum* secretes Myrotoxin B, which causes leaf spots in vegetables and *Pectobacterium carotovorum* leads to beet vascular necrosis and blackleg in potatoes and other vegetables [54].

Various endophytic microorganisms are essential in managing pest issues in orchids. Bacillus species, such as *Bacillus megaterium*, are known for their antimicrobial properties and can suppress pathogens while deterring pests like aphids, mealybugs, and fungal pathogens [55]. They also produce insecticidal toxins that reduce pest populations and improve orchid health. Similarly, *Pestalotiopsis* species, isolated from orchids like *Dendrobium*, possess antifungal and antioxidant properties, producing natural insecticidal metabolites that manage pests and enhance the plant's resistance to pathogens. *Trichoderma* species are well-regarded biocontrol agents that protect orchids by promoting plant growth and reducing susceptibility to root rot, thereby indirectly preventing pest infestations. Although some *Fusarium* species are pathogens, certain species produce bioactive metabolites that suppress pests such as nematodes and fungi. *Enterobacter* species, like *Enterobacter cloacae*, exhibit antimicrobial and insecticidal activity, releasing VOCs to repel pests and attract natural predators, while also promoting orchid growth. *Rhodotorula* species, a genus of yeast, produce antimicrobial metabolites and VOCs that repel herbivorous insects and attract predators, aiding in pest control [56]. These endophytes collectively enhance orchid health and contribute to effective pest management.

The orchid industry is rapidly expanding, with new varieties and hybrids developed to meet growing demand. Cymbidium mosaic virus poses a significant threat to orchids, as it is mechanically transmissible and quarantined in many countries. In a study, root endophyte *Piriformospora indica* combined with antiviral chemicals and defense-inducing compounds showed effective and practical method to control CymMV in Dendrobium orchids [57]. *Fusarium oxysporum* is a soil-borne fungal pathogen that causes various diseases in orchids, including wilt, root rot, sheath rot, leaf spots, and collar rot. It impairs water and nutrient transport, leading to wilting and plant death. Root rot results in poor root development and yellowing leaves, while sheath rot weakens the plant structure. Leaf spots can progress into blight, and collar rot affects the stem, causing plant collapse. These symptoms are common under conditions of high humidity, excess moisture, and poor drainage, leading to significant damage in orchid cultivation. A study investigated the culturable endophytic actinobacteria associated with ten epiphytic orchid species from Assam, India. A total of 51 morphologically distinct actinobacteria, mainly from the genus *Streptomyces* were isolated from surface-sterilized orchid roots and leaves. Two strains, *Streptomyces sp. VCLA3* and *Streptomyces sp. RVRA7*, showed significant damage to *Fusarium oxysporum* spores [58]. These research highlights the potential of endophytic microbes as sustainable alternatives to chemical fertilizers and pesticides, offering both plant growth benefits and broad-spectrum antimicrobial activity.

Mechanisms involved in abiotic and biotic stress resistance

Orchid endophytes employ various mechanisms to help their host plants cope with both abiotic and biotic stress. Endophytes help orchids combat biotic stress through several mechanisms. They produce osmolytes like proline to stabilize cellular structures and maintain hydration, enabling orchids to withstand stressors such as drought, salinity, and temperature fluctuations. Endophytes also synthesize antioxidants, such as phenolic compounds and flavonoids, which neutralize reactive oxygen species (ROS) generated under stress, thus protecting the plant from oxidative damage [59]. Additionally, they enhance nutrient uptake, especially in nutrient-poor environments, by solubilizing minerals like phosphate and producing growth-promoting substances like auxins and cytokinins. Endophytes also regulate plant hormones, including gibberellins and abscisic acid, to modulate stress responses, such as stomatal closure during drought. Endophytes help orchids combat biotic stress through several mechanisms. They activate the plant's defense system, triggering the production of defensive enzymes like chitinases, glucanases, and peroxidases, which protect against pathogens. Many endophytes also produce bioactive compounds, such as alkaloids, terpenoids, and antibiotics, which directly inhibit pathogen and pest growth, thereby preventing infections and minimizing damage. Additionally, some endophytes release VOCs that either repel herbivores or attract natural predators, reducing insect damage. Endophytes, including fungi and bacteria, can also directly antagonize pathogens by producing enzymes or metabolites that disrupt pathogen growth or by occupying niches that would otherwise be vulnerable to infection [60]. These mechanisms collectively enhance orchid resilience, contributing to better growth, survival, and reproduction in the face of environmental and biological stressors (Figure 3).

Conclusion

In conclusion, orchids are a diverse and ecologically important plant family, recognized for their distinct reproductive strategies and dependence on mycorrhizal fungi for seed germination and growth. Despite their advanced evolutionary traits, many orchid species face threats from habitat destruction, over-collection, and climate change, highlighting the need for conservation. Endophytic microorganisms, including fungi and bacteria, are crucial in boosting orchid resilience to both abiotic and biotic stresses. They enhance nutrient uptake, stress tolerance, and pathogen defense, promoting orchid health and productivity. Furthermore, these endophytes produce bioactive compounds that protect orchids from diseases and pests while boosting their medicinal value. Orchids being cosmopolitan, there are still numerous orchid species that need to be studied. As orchids are found even in extreme environments and have unique adaptation strategies for reproduction, understanding their interaction with endophytic microbes is necessary. New species of endophytic bacteria and fungi could be discovered that are significant in orchid lifecycle. These endophytes can be also utilized in producing industrially important enzymes and medicinally significant compounds. Furthermore, plant growth regulators can be industrially extracted from these endophytes that help in plant growth promotion, tolerance against stress conditions and immunity against various phytopathogens. Through mutualistic relationships, endophytes improve orchid survival, particularly in harsh conditions, emphasizing their role in conservation and sustainable orchid farming.

Methodology Used

This review was done with the help of comprehensive literature review survey targeting endophytic fungi and bacteria isolated from orchids. Peer-reviewed articles, reviews, and research papers published since the last ten years were retrieved through scientific databases such as PubMed, ScienceDirect, Scopus, and Google Scholar. Criteria for inclusion were derived from studies that reported:

- Isolation and characterization of endophytic microorganisms from different orchid species.
- Role of such microbes in orchid growth, development, and resistance to abiotic and biotic stress.
- Synthesis of plant growth regulators and secondary metabolites by endophytic microorganisms.
- Applications of endophytes in enhancing plant growth, stress resistance and conservation.

The identified research studies were studied for methodology, results, and conclusions with respect to microbial diversity, functional aspect, and biotechnological potential. Specific attention was paid to the research studies related to symbiotic germination, nutrient mobilization, and stress tolerance induced by endophytes. The review synthesizes the conclusions to highlight current trends, research gaps, and future directions.

Conflict of Interest

The authors declare that they have no conflicts of interest in the publication.

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