Review

Not peer-reviewed version

Cultivable Endophyte Resources in Medicinal Plants and Effects on Hosts

Yiming Wang , Yongjing Zhang , Hao Cong , Changgen Li , Jiaying Wu , Ludan Li , Jihong Jiang , Xiaoying Cao *

Posted Date: 27 June 2023

doi: 10.20944/preprints202306.1851.v1

Keywords: medicinal plants; endophyte; biocontrol; medicinal components

Review

Cultivable Endophyte Resources in Medicinal Plants and Effects on Hosts

Yiming Wang, Yongjing Zhang, Hao Cong, Changgen Li, Jiaying Wu, Ludan Li, Jihong Jiang and Xiaoying Cao *

The key laboratory of biotechnology for medicinal and edible plant resources of Jiangsu province, School of Life Sciences, Jiangsu Normal University, Xuzhou, Jiangsu Province 221116, China.

* Correspondence: cxy4868@jsnu.edu.cn (Xiaoying Cao)

Abstract: With the increasing demand for medicinal plants and the increasing shortage of resources, improving the quality and yield of medicinal plants and making more effective use of medicinal plants has become an urgent problem to be solved. In the process of growth and development of medicinal plants, due to the threat of various adversity, there are problems such as nutrient loss and yield decline. Using traditional chemical pesticides to control the stress resistance of plants will cause serious pollution to the environment and even endanger human health. Therefore, it is necessary to find suitable pesticide substitutes from natural ingredients. As an important part of the microecology of medicinal plants, endophytes can promote the growth of medicinal plants, improve the stress resistance of hosts, and promote the accumulation of active components of hosts. Because it has a more positive and direct impact on the host, and can metabolize rich medicinal ingredients, so researchers pay attention to it. This paper reviews the research in recent five years, aiming to provide ideas for improving the quality of medicinal plants, developing more microbial resources, exploring more medicinal natural products, and providing help for the development of the research on medicinal plants and endophytes.

Keywords: medicinal plants; endophyte; biocontrol; medicinal components

1. Introduction

Medicinal plants refer to plants used in medicine to prevent and treat diseases [1]. All or part of its plants are used for medicine and will also be used as raw materials for the pharmaceutical industry, which has a wide range of medicinal and economic values [2]. Especially in the field of traditional medicine represented by traditional Chinese medicine and Indian folk medicine, medicinal plants, as the main source of natural drugs, provide very important health care services for the population of developing countries[3–5]. With the rapid development of modern medicine, many clinical drugs still come from natural products extracted from medicinal plants [2]. Although many kinds of medicinal plants have been used in clinical treatment, due to environmental stress, overexploitation, low reproductive capacity, and other factors, some rare, high-demand, and wild medicinal plant resources can not meet the market demand, so how to improve the germplasm resources of medicinal plants has become an urgent problem to be solved.

However, in recent years, researchers have gradually realized that endophytes can play an important role in affecting the quality and yield of medicinal plants through special microbe-plant interactions [6,7]. Plant endophytes are microbial groups that widely exist in healthy medicinal plant tissues and coexist harmoniously with host plants, and will not cause significant damage to hosts [8]. They are also an important part of plant micro ecosystem. It is rich in species, mainly including endophytic fungi, endophytic bacteria and endophytic actinomycetes [7,9,10]. At present, endophytes have been isolated from a variety of medicinal plants, and many endophytes have been verified to secrete plant hormones, growth factors, etc., which are conducive to plant growth and development, and can also regulate the accumulation and production of active ingredients in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 June 2023 doi:10.20944/preprints202306.1851.v1

medicinal plants [10,11]. They increase the active ingredients of the host by producing the same or similar active products as the active ingredients in the host[11–13]. The most interesting thing is that these endophytes can convert the original active ingredients of the plant into new compounds. In 1993, Stierle et al isolated an endophytic fungus from *Taxus brevifolia* and found that it can produce paclitaxel, an anti-tumor substance similar to the host plant, which inspired researchers to find bioactive substances from endophytes of medicinal plants [14]. Endophytes provide a treasure house of new bioactive metabolites, especially alkaloids, saponins, quinones, flavonoids, terpenoids, etc., which have a lot of biological activities and have also become research hotspots in the composition and production of natural drugs [8].

In order to improve the quality of medicinal plants, we need to know more about the special relationship between endophytes and medicinal plants. In this paper, the cultivable endophyte sources and functions of Endophytes in medicinal plants were reviewed by searching the research in the past five years (2019-2023), and the development and utilization of endophytes in medicinal plants were prospected, to provide references for the development of endophyte products and improving the quality of medicinal plants.

2. Medicinal plants and their cultivable endophyte resources

2.1. Culturable endophytic bacteria diversity in medicinal plants

Atractylodes macrocephala, called Baizhu in Chinese, is a medicinal plant used in traditional Chinese medicine theoretical systems to treat gastrointestinal dysfunction, cancer, osteoporosis, obesity and other symptoms, and has various pharmacological activities. [15] Wu et al. [16] explored the cultivable endophytic bacteria in the stems, leaves, roots, and rhizomes of *Atractylodes macrocephala* in four different regions and their potential correlation with plant bioactives. A total of 118 endophytic bacteria belonging to 3 phyla, 5 classes, 11 orders, 26 families and 48 genera were identified from 4 *Atractylodes macrocephala* tissues. Among them, *Bacillus* sp. is the most widely distributed. Dendrobium is one of the largest genera of *Orchidaceae*, with more than 1500 species distributed all over the world [17]. As a medicinal plant, *Dendrobium* has contributed greatly to the medical industry by its anticancer, antifatigue, and gastrointestinal protective effects [18]. In addition, there are also many microbial resources in *Dendrobium*. Wang et al. [19] isolated and cultured endophytic bacteria from *Dendrobium officinale* samples of six different sources and cultivars. A total of 165 cultivable endophytic bacteria were isolated from sterilized *Dendrobium officinale* stems and classified into 43 species based on 16S rRNA gene sequence analysis. Mulberry, which belongs to the genus *Morus* of the *Moraceae* family, is an aggregated berry that is oval-shaped, palatable, and also rich in nutrients, it is regarded as a very important medicinal and edible plant due to its rich, effective chemical composition and wide range of biological activities[20–22]. Xu et al. [23] isolated a total of 608 endophytic bacteria from four mulberry cultivars, belonging to 4 phyla and 36 genera.

Bacteria, as the largest group of plant endophytes, have been isolated from many kinds of medicinal plants and widely studied due to their biocontrol functions[24–32]. By reviewing the recent literature, most of the endophytic bacteria of medicinal plants *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Agrobacterium* sp., etc., and a large number of endophytic bacteria exist in the roots, stems, and leaves, we collate some of the relevant data of the endophytic bacteria isolation as table 1.

Table 1. Endophytic bacteria resources isolated from medicinal plants in recent years.

Host plant	Tissue	Endophytic bacteria	Reference
Atractylodes macrocephala	Root, stem, leaf	*Bacillus* sp., *Rhodococcus* sp. *Mycobacterium* sp., *Pseudomonas* sp., *Mycolicibacterium* sp., *Leucobacter* sp., *Enterobacter* sp., *Rhizobium* sp., *Glutamicibacter* sp. and others, for a total of 58 genera.	[16]
Dendrobium	Stem	*Bacillus* sp., *Enterobacter* sp., *Klebsiella* sp., *Pantoea* sp., *Pseudomonas* sp., *Curtobacterium* sp.,	[19]

 doi:10.20944/preprints202306.1851.v1

		Burkholderia sp., *Microbacterium* sp., *Lysinibacillus* sp., and others, for a total of 23 genera.	
Mulberry	Stem	*Pantoea* sp., *Bacillus* sp., *Pseudomonas* sp., *Curtobacterium* sp., *Sphingomonas* sp. and others, for a total of 36 genera.	[23]
Marigold (*Calendula officinalis* L.)	Root, shoot	*Pantoea* sp., *Enterobacter* sp., *Pseudomonas* sp., *Achromobacter* sp., *Xanthomonas* sp., *Rathayibacter* sp., *Agrobacterium* sp., *Pseudoxanthomonas* sp., and *Beijerinckia* sp..	[24]
Camellia sinensis	Leaf, root	*Bacillus* sp., *Acinetobacter* sp., *Stenotrophomonas* sp., *Brevundimonas* sp., *Pseudomonas* sp., *Ochrobactrum* sp., *Alcaligenes* sp., and others, for a total of 16 genera.	[25]
Handroanthus impetiginosus	Leaf	*Bacillus* sp., *Paenibacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., *Rummeliibacillus* sp. and *Methylobacterium* sp..	[26]
European plum (*Prunus domestica*)	Shoot	*Pseudomonas* sp. and *Agrobacterium* sp.	[29]
Mint (*Endostemon obtusifolius*)	Leaf, root	*Paenibacillus* sp. etc.	[30]
Centella asiatica	Leaf	*Pseudomonas* sp., *Novosphingobium* sp., *Chryseobacterium* sp., *Enterobacter* sp., *Agrobacterium* sp., *Pantoea* sp. and *Paraburkholderia* sp.	[31]
Archidendron pauciflorum	Root, leaf, stem	*Bacillus* sp. etc.	[32]

2.2. Culturable endophytic fungal diversity in medicinal plants

Aconitum heterophyllum is an alkaloid rich medicinal plant that is widely used clinically in tradition Chinese medicine [33,34]. A total of 328 fungal isolates were found in leaf, stem and root tissues of plants by Hafeez et al., and identified 12 endophytic fungal species by molecular characterization [34]. *Crocus sativus* L. (family Iridaceae) has been widely used as an antimicrobial, antidepressant, digestive, anticancer, and anticonvulsant medicine due to its abundant natural products as well as antioxidant activity [35,36]. Lu et al. [37] isolated endophytic fungi from five different locations, five *Crocus sativus* different tissues (corm, scape, leaf, petal, and stigma) and identified a total of 32 endophytic fungal groups, assigned to 7 orders within 4 classes. Wang et al. [38] isolated 34 endophytic fungi from *Salvia miltiorrhiza*, a traditional Chinese medicine, belonging to 10 genera and 16 species. Ogbe et al. [30] isolated a total of 11 endophytic fungi from the roots and leaves of a drought tolerant mint species *Endostemon obtusifolius*. Similarly, five endophytic fungi were isolated from the leaf segments of wild *Dendrobium* nobile and identified as *Colletotrichum tropicicola*, *Fusarium keratoplasticum*, *Fusarium oxysporum*, *Fusarium solani*, and *Trichoderma longibrachiatum* [39]. Fan et al. [40] obtained 205 strains of endophytic fungi from the roots of *Codonopsis pilosula*, a traditional medicinal plant collected from six regions in Gansu Province, China, of which *Fusarium* sp., *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp., and *Plectosphaerella* sp. were the dominant genera. Niu et al. [41] isolated more than 30 kinds of Endophytic Fungi from the flowers of the medical plant *Vernonia anthelmintica.*

From the researches in recent years, it can be seen that *Fusarium* sp., *Aspergillus* sp., and *Penicillium* sp. can be isolated from most medicinal plants, and because of their many biological functions, they are regarded as the key research objects of endophytic fungi in medicinal plants. The recent research results are summarized in Table 2.

Table 2. Endophytic fungal resources isolated from medicinal plants in recent years.

Host plant	Tissue	Endophytic fungi	Reference
Aconitum heterophyllum	Root, stem, leaf	*Arthrinium* sp., *Chaetomium* sp., *Purpureocillium* sp., *Alternaria sp.*, *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., *and Bjerkandera* sp.	[34]
Crocus sativus	Corm, scape, leaf, petal, and stigma	*Penicillium* sp., *Sistotrema* sp., *and Bjerkandera* sp.	[37]
Salvia miltiorrhiza	Root	*Fusarium* sp., *Epicoccum* sp., *Aspergillus* sp., *Arthrinium* sp., *Coprinellus* sp., *Dictyosporium* sp., *Colletotrichum* sp., *Rhizoctonia* sp., *Phomopsis* sp. *and Pithomyces* sp.	[38]
Mint *(Endostemon obtusifolius)*	Root, leaf	*Fusarium* sp. etc.	[30]
Dendrobium	Leaf	*Colletotrichum* sp., *Fusariumand* sp., and *Trichoderma* sp.	[39]
Codonopsis pilosula	Root	*Fusarium* sp., *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp., *Plectosphaerella* sp. etc.	[40]
Vernonia anthelmintica	Flower	*Ovatospora* sp., *Chaetomium* sp., *Thielavia* sp. *and Aspergillus* sp.	[41]

2.3. Culturable endophytic actinomycetes diversity in medicinal plants

Dioscorea has powerful medicinal functions and is a potential source of bioactive substances for combating various diseases [42]. Zhou et al. [43] isolated 116 actinomycetes from *Dioscorea opposita* Thunb. tissue and found a new *Streptomyces* sp. with strong biocontrol function. As a traditional Chinese medicine, *Eucommia ulmoides* Oliv. has been used to treat various diseases since ancient times [44]. The research group led by Mo et al. [45,46] isolated two new species of *Nocardia* sp. from the leaves and roots of *Eucommia ulmoides* Oliv. Musa et al. [47] isolated 128 strains from the roots, stems and leaves of the Chinese medical herb *Thymus roseus* schipcz, with a predominence of *Streptomyces* sp., followed by *Nocardiopsis* sp., *Micrococcus* sp., *Kocuria* sp., and others. Salwan et al. [48] isolated Streptomyces with antibacterial activity from the unique medicinal plant *Viola odorata* in the Himalayas. Hu et al. [49] isolated two new Streptomyces strains from healthy leaves and seeds of *Xanthium sibiricum*.

According to the research on endophytic actinomycetes of medicinal plants in recent years, actinomycetes are mainly distributed in the roots of medicinal plants, and their number is greater than that of other tissues of plants. *Streptomyces* sp. is the main research object of actinomycetes, and *Streptomyces* sp. has received extensive attention because of its strong biological activity [50]. Actinomycetes of other genera can also be isolated from medicinal plants, but the number is relatively small compared with Streptomyces. Many studies have isolated new species of bioactive endophytic actinomycetes from medicinal plants, which greatly expanded microbial resources and laid a foundation for the industrial application of actinomycetes[45,46,49,51–53]. The results of endophyte isolation from medicinal plants in recent years are shown in Table 2.

Table 3. Endophytic actinomycete resources isolated from medicinal plants in recent years.

Host plant	Tissue	Endophytic actinomycetes	Reference
Dioscorea opposita		*Streptomyces* sp.	[43]
Eucommia ulmoides Oliv.	Roots, leaf	*Nocardia* sp.	[45,46]
Thymus roseus	Root, stem, leaf	*Nocardiopsis sp., Micrococcus sp., Kocuria sp.*, and etc.	[47]
Viola odorata	Root	*Streptomyces* sp.	[48]

Xanthium sibiricum	Leaf, seed	*Streptomyces* sp.	[49]
Kandelia candel	Root	*Nocardioides* sp.	[51]
Mentha haplocalyx	Bark	*Nakamurella* sp.	[52]
Acacia mangium	Root	*Fodinicola* sp.	[53]

3. Beneficial effects of Endophytes from medicinal plants on the host

There are abundant endophytes in medicinal plants. In recent years, many studies have shown that endophytes have made important contributions in promoting the growth of medicinal plants, enhancing the stress resistance of medicinal plants and biological control of plant diseases.

3.1. Promoting the growth of medicinal plants

Khan et al. [54] isolated an endophytic fungus *Acremonium* sp. Ld-03, which has antibacterial activity and can produce IAA and siderophores, from the medicinal plant *Lilium davidii*. Application of 40% culture dilution of *Acremonium* sp., the root and bud length of *Allium tuberosum* can be significantly increased. Zou et al. [55] isolated a *Bacillus subtilis* strain from the medicinal plant *Aconitum carmichaelii* DEBX., which can produce gluconase, cellulase, protease, indole acid, siderophore, antifungal lipopeptides and polyketides, and significantly increased the fresh weight and dry weight of the host stem, main root and lateral root. Tao et al. [56] isolated four strains of endophytic bacteria with indole acid production, phase solubilization, and nitrogen fixation abilities from the precious traditional Chinese medicine *Pairs polyphylla* var. *yunnanensis*, and significantly increased the host's biomass. The metabolite of endogenous *Aspergillus niger* isolated from *Albizia lebbeck* (L.) Benth by Mathur et al., effectively promoted seed germination of many plants [57]. Purushotham et al. [58] isolated an endophytic actinomycete, *Nocardia* sp. TP1BA1B, which can solubilize phosphate and produce siderophores from New Zealand native medical plant *Pseudowitera colorata* (horopito), can promote the growth of seedlings.

The growth of medicinal plants is related to various environmental factors, such as light, temperature and microorganisms, among which endophytes are very important environmental factors to participate in the growth process of the host [59]. Endophytes promote the growth and development of plants in different ways, such as secreting iron carriers to improve the utilization rate of iron in plants. Endophytes with nitrogen fixation, phosphate solubilization and potassium solubilization promote the growth of medicinal plants by promoting the absorption of nitrogen, phosphorus and potassium. Endophytes can also promote plant growth by providing growth hormone to the host [10,60,61].

3.2. Enhance the stress resistance of medicinal plants

Li et al. [62] isolated a strain of *Streptomyces* from *Glycyrrhiza uralensis* and confirmed through inoculation that this strain can enhance the strain can enhance the tolerance of the host to drought, salt and drought salt conditions. Studies have found that under drought stress, the growth of *Helianthus tuberosus* L. (Jerusalem artichoke) can be better promoted by the inoculation of endophytic bacteria [63]. *Sphingomonas paucimobilis*, an endophytic bacterium in the rare medicinal plant *Dendrobium officinale*, has good resistance to stresses of salt, drop and cadmium, and this strain is the only one with birth ability reported in this species [64]. Some researchers isolated Endophytes from *Astragalus mongholicus* and co-inoculated them with *Trichoderma* Strains under drought conditions, which significantly improved the root biomass, root length, calycosin-7-O-β-D-glucoside content of the host, and activities of nitrate reductase and soil urease [65].

Endophytes can enhance the environmental adaptability of host plants by enhancing the expression of stress resistance related genes in host plants and increasing the activity of related enzymes [66]. In addition, some endophytes may also produce antibiotic compounds, antimicrobial peptides or alkaloids to help the host resist pests and diseases [67,68].

3.3. Promoting the accumulation of secondary metabolites in medicinal plants

Endophytic fungus *Cladosporium tenuissimum* DF11 isolated from *Salvia miltiorrhiza* by Chen et al. [69], promoted the biosynthesis and accumulation of Tanshinone in roots by up regulating the expression of HMGR, DXS, DXR, GGPPS, CPS, KSL and CYP76AH1, which are key enzyme genes of tanshinone biosynthesis pathway. Other researchers used endophytes of *Salvia miltiorrhiza* to prepare elicitors, which affected the accumulation of metabolites in hairy roots of *Salvia miltiorrhiza* by inducing the expression of key genes (SmAACT, SmGGPPS, and SmPAL) [70]. Researchers have also isolated two strains of fungi from *Salvia abrotanoides* that can increase host cryptotanshinone and tanshinone IIA production [71]. Ye et al. [72] isolated three Endophytes from *houttuynia cordata*, named *ilyonectria liriodendra*, unidentified fungal sp., and *Penicillium citrinum*, which can respectively increase the phenolic compounds of the host, increase the components such as afzelin, decanal, 2-undecanone, and increase the biomass of the host. Xie et al. [73] isolated an endophytic fungus *Schizophyllum commune* from *Panax ginseng* and significantly enhanced the expression of key enzyme genes involved in ginsenoside biosynthesis pathways such as pgHMGR, pgSS, pgSE, and pgSD under co-culture conditions, promoting the accumulation of specific ginsenosides.

Endophytes can directly participate in the synthesis of secondary metabolites of medicinal plants, and can also induce the formation of secondary metabolites of medicinal plants [74]. Selecting appropriate endophytes to act on medicinal plants can improve the content of secondary metabolites, which is of great significance for improving the quality of medicinal plants, protecting endangered medicinal plants, and synthesizing and developing new drugs.

3.4. Helping the host resist pathogens

Streptomyces dioscori isolated from *Glycyrrhiza uralensis* exhibited inhibitory effects on three pathogenic fungi: *Rhizoctonia solani*, *Fusarium acuminatum*, and *Sclerotinia scrotiorum* [62]. An endophytic fungus, *Diaporthe* sp., was isolated from the leaves of the Indian medicinal plant *Chloranthus elator* Sw., and its camphor odor volatiles showed inhibitory effects on eight fungal pathogens in vitro [75]. *Burkholderia gladioli*, an endophytic bacterium from *Crocus sativus* Linn., can reduce corm rot and increase endogenous jasmonic acid (JA) level and expression of JA-regulated and other plant defence genesthrough antibacterial effect, and improve the host's resistance to *Fusarium oxysporum* [76].

Many endophytes can inhibit the occurrence of plant diseases caused by pathogenic bacteria. Endophytes can inhibit the activity of pathogens by inducing host resistance to resist the infection of pathogens and competing with pathogenic bacteria to produce antibiotics, hydrolytic enzymes, alkaloids and other secondary metabolites and signal interference, to resist the disease caused by pathogens in host plants [77].

4. Medicinal components produced by endophytes in medicinal plants

An endophytic fungus *Xylaria feejeensis*, derived from the medicinal plant *Geophila repens* in Sri Lanka. Integrated acids, derived from fungal metabolites, has strong antibacterial activity and is a potential resource of antibiotics [78]. Saikosaponin d (SSd) is an important medicinal component of the medicinal plant *Bupleurum scorzonerifolium* Willd.. Some researchers isolated two endophytic Fungi from *Bupleurum scorzonerifolium* Willd., which can produce saikosaponin through UPLC/Q-TOF-MS detection [79]. The metabolite 7-methoxy-13-dehydroxypaxilline of *Penicillium* sp., an endophytic fungus isolated from the leaves of the traditional medicinal plant *Baphicacanthus cusia* (Nees) bremek., is a new indole diterpenoid, which exhibits anticancer activity [80]. Gu et al. [81] isolated a new compound phomopolide G from the fermentation broth of endophytic fungi of *Artemisia argyi* and showed a wide range of antibacterial activities. *Cochliobolus* sp., an endophytic fungus of the Indian medical herb *Andrographis paniculata*, can metabolize the alkaloid aziridine, 1-(2-aminoethyl) -, and can be antibacterial and insect resistant [82]. There is a new crystalline compound 5- (1-hydroxybutyl) - 4-methoxy-3-methyl-2h- pyran-2-one (c-hmmp) in the endophytic fungus *Colletotrichum acutatum* in the medicinal plant *Angelica sinensis* and shows antibacterial, antimalarial, anticancer, antioxidant and other activities [83]. The antibacterial compound 1,4-dihydroxy-2-methyl-anthraquinone was also isolated from the endophytic bacteria of *archidendron pauciflorum* [32]. The structures of the above compounds are shown in Figure 1.

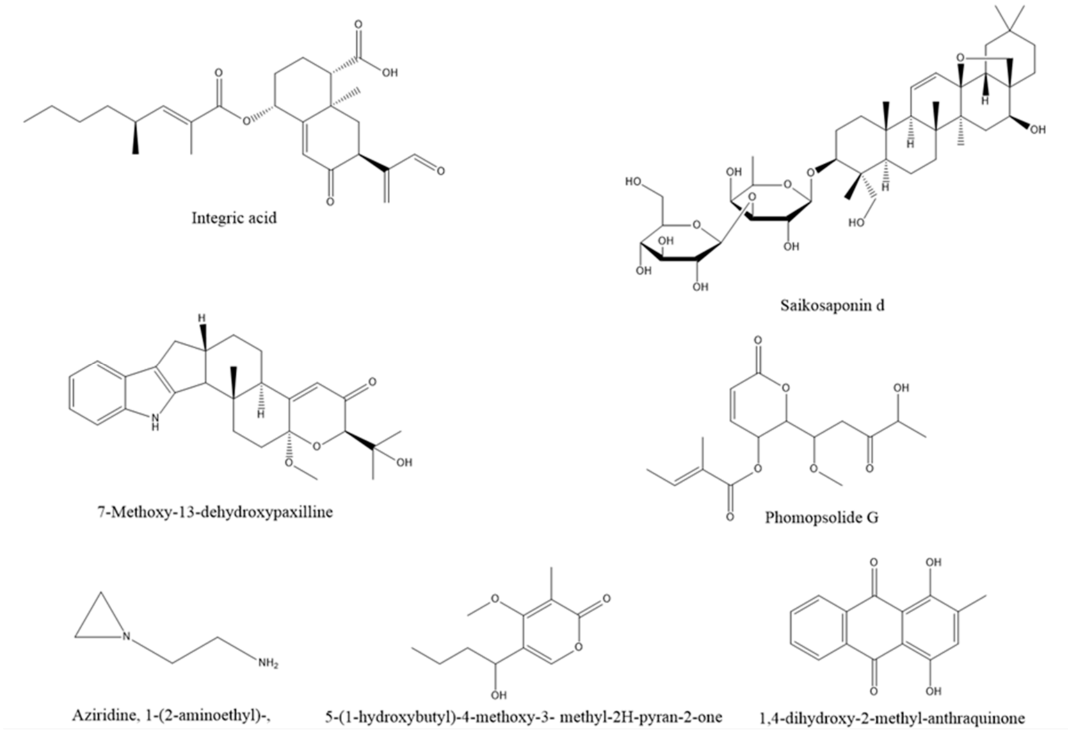

Figure 1. Some active ingredients from endophytes of medicinal plants**.**

At present, many alkaloids, flavonoids, phenolic acids, terpenoids, coumarins and other substances with antioxidant activity have been isolated from endophytes and their secondary metabolites of medicinal plants**[10,11,32,84–86]**. These natural antioxidant active substances often have anti-inflammatory, antioxidant, antibacterial, anti-tumor, Antiviral and other functions [10,11,87]. Through searching the literature in the past five years, it was found that the bioactive natural products of Endophytes in medicinal plants were metabolized by fungi, while the metabolites of bacteria and actinomycetes were mainly antibiotics [10]. More and more studies have found that the 'Phytochemistry components' produced by host plants may be produced by or closely related to endophytes. More and more studies have found that natural product produced by host plants may be produced by endophytes or closely related to endophytes [88]. Further research on the metabolites of endophytes will be of great significance for the development of medicinal plants and clinical drugs.

5. Discussion

In recent years, people have gradually realized that endophytes play an important role in affecting the yield and quality of crude drugs by interacting with the host in a specific way. The traditional method of endophyte research is to use the artificial medium to culture, isolate and purify microorganisms to obtain pure culture strains, and use microscopic technology to observe and classify their morphology (Figure 2). According to the physiological and biochemical characteristics of the strain, 16S rRNA, its gene sequencing, and other molecular biological methods were used for gene identification [10].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 June 2023 doi:10.20944/preprints202306.1851.v1

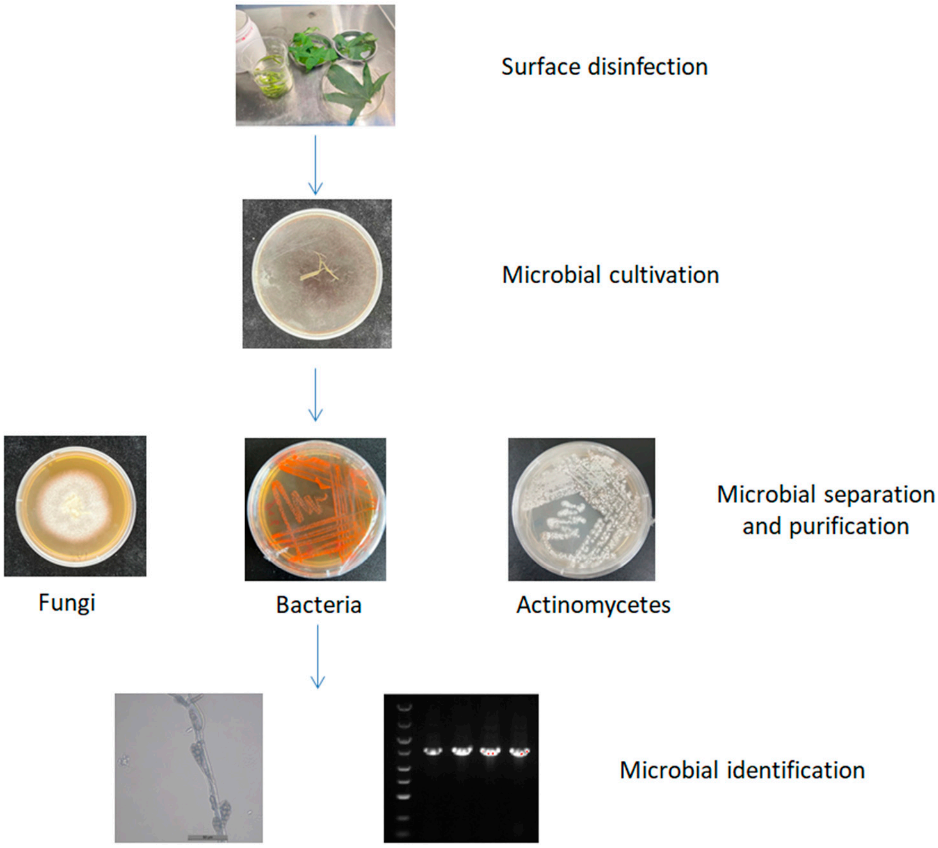

Figure 2. Traditional endophyte research process.

The rapid development of gene sequencing technology, especially the emergence of high-throughput sequencing and other technical means, has brought unprecedented development to microbiology research [89]. High throughput sequencing technology has been applied to the study of the structure and diversity of a variety of plant endophytes, and avoids the process of endophyte culture, to explore more microbial resources [89,90]. Some researchers conducted 16S rRNA gene based high-throughput sequencing analysis on the root samples of coastal native salt marsh plants, and detected a total of 4 Actinobacteria, 14 orders, 35 families, and 63 known genera, mainly including *Arthrobacter* sp., *Mycobacterium* sp., *Micromonospora* sp., *Nocardia* sp., and *Streptomyces* sp. [91]. Pan et al. also used the method of Illumina HiSeq sequencing technology to explore the composition and diversity of Endophytes in the roots, stems and leaves of *Huperzia serrata*. Total effective reads were assigned to 936 operational taxonomic units, belonging to 12 phylla and 289 general. *Sphingomonas* sp., *Acidibacter* sp., *Bradyrhizobium* sp., *Bryobacter* sp., *Methylocella* sp., *Nocardioides* sp., etc. were the dominant genera, and the microbial enrichment in roots was significantly higher than that in stems and leaves [92]. High throughput sequencing technology can explore the richness of microbial resources in medicinal plants, but only a small part can be isolated, which also means that there is still a great research potential for endophytes in medicinal plants. Therefore, it is necessary to optimize cultivation methods to obtain more cultivable microbial resources. Medicinal plants are the foundation of the development of pharmaceutical industry. With the understanding and utilization of the cultivation, growth and various functions of medicinal plants, the quality of medicinal plants has attracted great attention in society [93]. The quality and yield of medicinal plant raw materials are largely affected by many factors, such as plant genetic background, ecological habitat of plants and soil nutrients [94,95]. Endophytes live in medicinal plants and have the functions of promoting the growth of host plants, enhancing the stress resistance of host plants, and regulating the synthesis of secondary metabolites with important medicinal value [96]. Rational utilization of microbial resources, especially endophyte resources, is conducive to

 doi:10.20944/preprints202306.1851.v1

biological control and bioremediation of contaminated soil [97,98], thus reducing the use of chemical fertilizers and pesticides, which is of great significance for sustainable development.

The secondary metabolites of Endophytes from most medicinal plants have medicinal activities, which have great potential in the development of new drugs [11,98]. If endophytes of medicinal plants can be used to produce drugs in the future, it will make up for the demand for some rare medicinal plants to a certain extent [99]. The research methods of secondary metabolites of plant endophytes are usually based on the separation and purification strategy of natural products after endophyte fermentation. However, when the endophyte leaves the host, its biosynthesis of metabolites during fermentation is not as efficient as when it is in the host, and the microbial metabolic ability and biological function will decline after multiple generations of cultivation [100]. Therefore, optimizing the culture method of endophytes, promoting the metabolism of endophytes of medicinal plants, producing more metabolites with medicinal effects, and maintaining the biological function of endophytes are also urgent problems to be solved.

The use of chemical pesticides causes serious pollution to the environment and even endangers human health [101], so it is necessary to put forward more sustainable development strategies for the environment and human health, and find suitable pesticide substitutes. Endophytes come from plants and act on plants, and they are rich in species and have strong biological control functions [56,102]. Therefore, they are the most suitable to replace chemical pesticides in the cultivation and protection of medicinal plants, and are an important environmental protection strategy. The metabolites of Endophytes in medicinal plants are also a huge treasure house for the discovery of medicinal ingredients, which can greatly make up for the shortage of natural resources, which also makes the research of endophytes attract more researchers' attention [8,103]. In order to fully develop the research and application of Endophytes in medicinal plants, the following problems need to be solved urgently: 1. How to make the biocontrol strains survive in the environment outside the plant for a long time? 2. Can the reduced metabolic capacity and biological function of endophytes after multi-generation culture be overcome by optimizing culture regulation? 3. In vitro endophytes can produce a variety of secondary metabolites to antagonize pathogenic bacteria and inhibit their growth. Can the development of new green pesticides be produced in large quantities with high efficiency? 4. How can we improve the fermentation efficiency of Endophytes in medicinal plants, to tap more abundant potential medicinal ingredients? To solve the above problems, exploring the interaction mechanism between endophytic bacteria and hosts, as well as the metabolic mechanism of medicinal components, and conducting more research on the application of endophytes in production, will be of great help to the production of medicinal plants and the development of medicinal components.

Author Contributions: Conceptualization, J.J.and X.C.; methodology, X.C.; software, Y.W.; formal analysis, X.C.; investigation, J.J.; resources, J.J.; writing—original draft preparation, Y.W.; writing—review and editing, Y.W, X.C.,Y.Z.,H.C, L.L and J.W.; visualization, C.L.; supervision, J.J.; project administration, J.J.; funding acquisition, J.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (Grant No. 31770613) and the postgraduate research and practice innovation program of Jiangsu province (Grant No. KYCX22_2822).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aye, M.M.; Aung, H.T.; Sein, M.M.; Armijos, C. A Review on the Phytochemistry, Medicinal Properties and Pharmacological Activities of 15 Selected Myanmar Medicinal Plants. *Molecules* 2019, *24*, doi:10.3390/molecules24020293.

2. Tang, P.; Shen, T.; Wang, H.; Zhang, R.; Zhang, X.; Li, X.; Xiao, W. Challenges and opportunities for improving the druggability of natural product: Why need drug delivery system? *Biomed Pharmacother* 2023, *164*, 114955, doi:10.1016/j.biopha.2023.114955.
3. Zhang, Y.L.; Wang, Y.L.; Yan, K.; Deng, Q.Q.; Li, F.Z.; Liang, X.J.; Hua, Q. Nanostructures in Chinese herbal medicines (CHMs) for potential therapy. *Nanoscale Horiz* 2023, doi:10.1039/d3nh00120b.
4. Choudhary, A.; Noman, M.; Bano, U.; Akhtar, J.; Shaikh, Y.; Yar, M.S. Global uses of traditional herbs for hepatic diseases and other pharmacological actions: A comprehensive review. *Polim Med* 2023, doi:10.17219/pim/165977.
5. Wu, S.; Wang, C.; Bai, D.; Chen, N.; Hu, J.; Zhang, J. Perspectives of international multi-center clinical trials on traditional Chinese herbal medicine. *Front Pharmacol* 2023, *14*, 1195364, doi:10.3389/fphar.2023.1195364.
6. Rani, S.; Kumar, P.; Dahiya, P.; Maheshwari, R.; Dang, A.S.; Suneja, P. Endophytism: A Multidimensional Approach to Plant-Prokaryotic Microbe Interaction. *Front Microbiol* 2022, *13*, 861235, doi:10.3389/fmicb.2022.861235.
7. Choudhary, M.; Gupta, S.; Dhar, M.K.; Kaul, S. Endophytic Fungi-Mediated Biocatalysis and Biotransformations Paving the Way Toward Green Chemistry. *Front Bioeng Biotechnol* 2021, *9*, 664705, doi:10.3389/fbioe.2021.664705.
8. Gouda, S.; Das, G.; Sen, S.K.; Shin, H.S.; Patra, J.K. Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. *Front Microbiol* 2016, *7*, 1538, doi:10.3389/fmicb.2016.01538.
9. Nalini, M.S.; Prakash, H.S. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. *Lett Appl Microbiol* 2017, *64*, 261-270, doi:10.1111/lam.12718.
10. Tshikhudo, P.P.; Ntushelo, K.; Mudau, F.N. Sustainable Applications of Endophytic Bacteria and Their Physiological/Biochemical Roles on Medicinal and Herbal Plants: Review. *Microorganisms* 2023, *11*, doi:ARTN 453 10.3390/microorganisms11020453.
11. Hashem, A.H.; Attia, M.S.; Kandil, E.K.; Fawzi, M.M.; Abdelrahman, A.S.; Khader, M.S.; Khodaira, M.A.; Emam, A.E.; Goma, M.A.; Abdelaziz, A.M. Bioactive compounds and biomedical applications of endophytic fungi: a recent review. *Microb Cell Fact* 2023, *22*, 107, doi:10.1186/s12934-023-02118-x.
12. Zhang, J.; Zhu, Y.H.; Si, J.P.; Wu, L.S. Metabolites of medicine food homology-derived endophytic fungi and their activities. *Curr Res Food Sci* 2022, *5*, 1882-1896, doi:10.1016/j.crfs.2022.10.006.
13. Tripathi, A.; Pandey, P.; Tripathi, S.N.; Kalra, A. Perspectives and potential applications of endophytic microorganisms in cultivation of medicinal and aromatic plants. *Front Plant Sci* 2022, *13*, 985429, doi:10.3389/fpls.2022.985429.
14. Stierle, A.; Strobel, G.; Stierle, D. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. *Science* 1993, *260*, 214-216, doi:10.1126/science.8097061.
15. Zhu, B.; Zhang, Q.L.; Hua, J.W.; Cheng, W.L.; Qin, L.P. The traditional uses, phytochemistry, and pharmacology of Atractylodes macrocephala Koidz.: A review. *J Ethnopharmacol* 2018, *226*, 143-167, doi:10.1016/j.jep.2018.08.023.
16. Wu, W.; Wang, S.; Wu, J.; He, B.; Zhu, B.; Qin, L. Influence of tissue and geographic locality on culturable endophytic bacteria of Atractylodes macrocephala. *Microbiology (Reading)* 2021, *167*, doi:10.1099/mic.0.001109.
17. Wang, Z.; Zhao, M.; Cui, H.; Li, J.; Wang, M. Transcriptomic Landscape of Medicinal Dendrobium Reveals Genes Associated With the Biosynthesis of Bioactive Components. *Front Plant Sci* 2020, *11*, 391, doi:10.3389/fpls.2020.00391.
18. Wu, W.; Lin, Y.; Farag, M.A.; Li, Z.; Shao, P. Dendrobium as a new natural source of bioactive for the prevention and treatment of digestive tract diseases: A comprehensive review with future perspectives. *Phytomedicine* 2023, *114*, 154784, doi:10.1016/j.phymed.2023.154784.
19. Wang, S.S.; Liu, J.M.; Sun, J.; Sun, Y.F.; Liu, J.N.; Jia, N.; Fan, B.; Dai, X.F. Diversity of culture-independent bacteria and antimicrobial activity of culturable endophytic bacteria isolated from different Dendrobium stems. *Sci Rep* 2019, *9*, 10389, doi:10.1038/s41598-019-46863-9.
20. Yuan, Q.; Zhao, L. The Mulberry (Morus alba L.) Fruit-A Review of Characteristic Components and Health Benefits. *J Agric Food Chem* 2017, *65*, 10383-10394, doi:10.1021/acs.jafc.7b03614.
21. Kang, C.-W.; Park, M.; Lee, H.-J. Mulberry (Morus alba L.) Leaf Extract and 1-Deoxynojirimycin Improve Skeletal Muscle Insulin Resistance via the Activation of IRS-1/PI3K/Akt Pathway in db/db Mice. *Life* 2022, *12*, 1630.
22. Hao, J.; Gao, Y.; Xue, J.; Yang, Y.; Yin, J.; Wu, T.; Zhang, M. Phytochemicals, Pharmacological Effects and Molecular Mechanisms of Mulberry. *Foods* 2022, *11*, doi:10.3390/foods11081170.
23. Xu, W.; Wang, F.; Zhang, M.; Ou, T.; Wang, R.; Strobel, G.; Xiang, Z.; Zhou, Z.; Xie, J. Diversity of cultivable endophytic bacteria in mulberry and their potential for antimicrobial and plant growth-promoting activities. *Microbiol Res* 2019, *229*, 126328, doi:10.1016/j.micres.2019.126328.
24. Shurigin, V.; Alaylar, B.; Davranov, K.; Wirth, S.; Bellingrath-Kimura, S.D.; Egamberdieva, D. Diversity and biological activity of culturable endophytic bacteria associated with marigold (Calendula officinalis L.). *AIMS Microbiol* 2021, *7*, 336-353, doi:10.3934/microbiol.2021021.

25. Hazarika, S.N.; Saikia, K.; Borah, A.; Thakur, D. Prospecting Endophytic Bacteria Endowed With Plant Growth Promoting Potential Isolated From Camellia sinensis. *Front Microbiol* 2021, *12*, 738058, doi:10.3389/fmicb.2021.738058.
26. Yarte, M.E.; Gismondi, M.I.; Llorente, B.E.; Larraburu, E.E. Isolation of endophytic bacteria from the medicinal, forestal and ornamental tree Handroanthus impetiginosus. *Environ Technol* 2022, *43*, 1129-1139, doi:10.1080/09593330.2020.1818833.
27. Borah, A.; Das, R.; Mazumdar, R.; Thakur, D. Culturable endophytic bacteria of Camellia species endowed with plant growth promoting characteristics. *J Appl Microbiol* 2019, *127*, 825-844, doi:10.1111/jam.14356.
28. de Oliveira, A.A.; Ramalho, M.O.; Moreau, C.S.; Campos, A.E.C.; Harakava, R.; Bueno, O.C. Exploring the diversity and potential interactions of bacterial and fungal endophytes associated with different cultivars of olive (Olea europaea) in Brazil. *Microbiol Res* 2022, *263*, 127128, doi:10.1016/j.micres.2022.127128.
29. Kolytaite, A.; Vaitiekunaite, D.; Antanyniene, R.; Baniulis, D.; Frercks, B. Monilinia fructigena Suppressing and Plant Growth Promoting Endophytic Pseudomonas spp. Bacteria Isolated from Plum. *Microorganisms* 2022, *10*, doi:10.3390/microorganisms10122402.
30. Ogbe, A.A.; Gupta, S.; Stirk, W.A.; Finnie, J.F.; Van Staden, J. Growth-Promoting Characteristics of Fungal and Bacterial Endophytes Isolated from a Drought-Tolerant Mint Species Endostemon obtusifolius (E. Mey. ex Benth.) N. E. Br. *Plants (Basel)* 2023, *12*, doi:10.3390/plants12030638.
31. Mahlangu, S.G.; Tai, S.L. Morphological and molecular characterization of bacterial endophytes from Centella asiatica leaves. *J Genet Eng Biotechnol* 2022, *20*, 171, doi:10.1186/s43141-022-00456-8.
32. Priyanto, J.A.; Prastya, M.E.; Astuti, R.I.; Kristiana, R. The Antibacterial and Antibiofilm Activities of the Endophytic Bacteria Associated with Archidendron pauciflorum against Multidrug-Resistant Strains. *Appl Biochem Biotechnol* 2023, doi:10.1007/s12010-023-04382-4.
33. Punia, A.; Joshi, R.; Kumar, R. Identification and quantification of eight alkaloids in Aconitum heterophyllum using UHPLC-DAD-QTOF-IMS: A valuable tool for quality control. *Phytochem Anal* 2022, *33*, 1121-1134, doi:10.1002/pca.3164.
34. Hafeez, S.; Yaqoob, S.; Magray, A.R.; Kamili, A.N.; Ganai, B.A. Molecular characterization of fungal endophyte diversity isolated from Aconitum heterophyllum: a critically endangered medicinal plant of Kashmir Himalaya. *Int Microbiol* 2023, doi:10.1007/s10123-023-00331-7.
35. Matraszek-Gawron, R.; Chwil, M.; Terlecki, K.; Skoczylas, M.M. Current Knowledge of the Antidepressant Activity of Chemical Compounds from Crocus sativus L. *Pharmaceuticals (Basel)* 2022, *16*, doi:10.3390/ph16010058.
36. Maqbool, Z.; Arshad, M.S.; Ali, A.; Aziz, A.; Khalid, W.; Afzal, M.F.; Bangar, S.P.; Addi, M.; Hano, C.; Lorenzo, J.M. Potential Role of Phytochemical Extract from Saffron in Development of Functional Foods and Protection of Brain-Related Disorders. *Oxid Med Cell Longev* 2022, *2022*, 6480590, doi:10.1155/2022/6480590.
37. Lu, J.; Wang, J.; Zhang, J.; Zhu, Y.; Qin, L.; Zhu, B. Diversity of Culturable Endophytic Fungi in Crocus sativus and Their Correlation with Crocin Content. *Curr Microbiol* 2023, *80*, 73, doi:10.1007/s00284-023-03177-4.
38. Wang, G.K.; Yang, J.S.; Huang, Y.F.; Liu, J.S.; Tsai, C.W.; Bau, D.T.; Chang, W.S. Culture Separation, Identification and Unique Anti-pathogenic Fungi Capacity of Endophytic Fungi from Gucheng Salvia Miltiorrhiza. *In Vivo* 2021, *35*, 325-332, doi:10.21873/invivo.12263.
39. Sarsaiya, S.; Jain, A.; Jia, Q.; Fan, X.; Shu, F.; Chen, Z.; Zhou, Q.; Shi, J.; Chen, J. Molecular Identification of Endophytic Fungi and Their Pathogenicity Evaluation Against Dendrobium nobile and Dendrobium officinale. *Int J Mol Sci* 2020, *21*, doi:10.3390/ijms21010316.
40. Fan, L.; Li, Y.; Wang, X.; Leng, F.; Li, S.; Zhu, N.; Chen, K.; Wang, Y. Culturable endophytic fungi community structure isolated from Codonopsis pilosula roots and effect of season and geographic location on their structures. *BMC Microbiol* 2023, *23*, 132, doi:10.1186/s12866-023-02848-3.
41. Niu, L.; Rustamova, N.; Ning, H.; Paerhati, P.; Lu, C.; Yili, A. Diversity and Biological Activities of Endophytic Fungi from the Flowers of the Medicinal Plant Vernonia anthelmintica. *Int J Mol Sci* 2022, *23*, doi:10.3390/ijms231911935.
42. Wang, Z.; Zhao, S.; Tao, S.; Hou, G.; Zhao, F.; Tan, S.; Meng, Q. Dioscorea spp.: Bioactive Compounds and Potential for the Treatment of Inflammatory and Metabolic Diseases. *Molecules* 2023, *28*, doi:10.3390/molecules28062878.
43. Zhou, S.; Zhou, Y.; Li, C.; Wu, W.; Xu, Y.; Xia, W.; Huang, D.; Huang, X. Identification and genomic analyses of a novel endophytic actinobacterium Streptomyces endophytica sp. nov. with potential for biocontrol of yam anthracnose. *Front Microbiol* 2023, *14*, 1139456, doi:10.3389/fmicb.2023.1139456.
44. Huang, L.; Lyu, Q.; Zheng, W.; Yang, Q.; Cao, G. Traditional application and modern pharmacological research of Eucommia ulmoides Oliv. *Chin Med* 2021, *16*, 73, doi:10.1186/s13020-021-00482-7.
45. Mo, P.; Chen, Y.; Zou, F.; Zhou, J.; Zou, W.; Gao, J. Nocardiopsis eucommiae sp. nov., a novel endophytic actinomycete isolated from leaves of Eucommia ulmoides Oliv. *Int J Syst Evol Microbiol* 2022, *72*, doi:10.1099/ijsem.0.005654.

46. Mo, P.; Li, K.; Zhou, J.; Zhou, F.; He, J.; Zou, W.; Gao, J. Nocardiopsis changdeensis sp. nov., an endophytic actinomycete isolated from the roots of Eucommia ulmoides Oliv. *J Antibiot (Tokyo)* 2023, *76*, 191-197, doi:10.1038/s41429-023-00596-0.
47. Musa, Z.; Ma, J.; Egamberdieva, D.; Abdelshafy Mohamad, O.A.; Abaydulla, G.; Liu, Y.; Li, W.J.; Li, L. Diversity and Antimicrobial Potential of Cultivable Endophytic Actinobacteria Associated With the Medicinal Plant Thymus roseus. *Front Microbiol* 2020, *11*, 191, doi:10.3389/fmicb.2020.00191.
48. Salwan, R.; Rana, A.; Saini, R.; Sharma, A.; Sharma, M.; Sharma, V. Diversity analysis of endophytes with antimicrobial and antioxidant potential from Viola odorata: an endemic plant species of the Himalayas. *Braz J Microbiol* 2023, doi:10.1007/s42770-023-01010-5.
49. Hu, S.; Wang, Y.; Wang, J.; Liu, K.; Tang, X.; Gao, J. Streptomyces xanthii sp. nov. and Streptomyces roseirectus sp. nov. isolated from a Chinese medicinal plant. *Int J Syst Evol Microbiol* 2021, *71*, doi:10.1099/ijsem.0.004962.
50. Vurukonda, S.S.K.P.; Giovanardi, D.; Stefani, E. Plant Growth Promoting and Biocontrol Activity of Streptomyces spp. as Endophytes. *International Journal of Molecular Sciences* 2018, *19*, doi:ARTN 952
10.3390/ijms19040952.
51. Chen, M.S.; Chen, F.; Chen, X.H.; Zheng, Z.Q.; Ma, X.; Tuo, L. Nocardioides mangrovi sp. nov., a novel endophytic actinobacterium isolated from root of Kandelia candel. *Int J Syst Evol Microbiol* 2022, *72*, doi:10.1099/ijsem.0.005295.
52. Yan, X.R.; Chen, M.S.; Yang, C.; An, M.B.; Li, H.Y.; Shi, H.C.; Tuo, L. Nakamurella flava sp. nov., a novel endophytic actinobacterium isolated from Mentha haplocalyx Briq. *Int J Syst Evol Microbiol* 2020, *70*, 835-840, doi:10.1099/ijsem.0.003831.
53. Pham, H.T.T.; Suwannapan, W.; Koomsiri, W.; Inahashi, Y.; Take, A.; Matsumoto, A.; Thamchaipenet, A. Fodinicola acaciae sp. nov., an Endophytic Actinomycete Isolated from the Roots of Acacia mangium Willd. and Its Genome Analysis. *Microorganisms* 2020, *8*, doi:10.3390/microorganisms8040467.
54. Khan, M.S.; Gao, J.; Munir, I.; Zhang, M.; Liu, Y.; Moe, T.S.; Xue, J.; Zhang, X. Characterization of Endophytic Fungi, Acremonium sp., from Lilium davidii and Analysis of Its Antifungal and Plant Growth-Promoting Effects. *Biomed Res Int* 2021, *2021*, 9930210, doi:10.1155/2021/9930210.
55. Zou, L.; Wang, Q.; Wu, R.; Zhang, Y.; Wu, Q.; Li, M.; Ye, K.; Dai, W.; Huang, J. Biocontrol and plant growth promotion potential of endophytic Bacillus subtilis JY-7-2L on Aconitum carmichaelii Debx. *Front Microbiol* 2022, *13*, 1059549, doi:10.3389/fmicb.2022.1059549.
56. Tao, L.; Qiuhong, L.; Fuqiang, Y.; Shuhui, Z.; Suohui, T.; Linyuan, F. Plant growth-promoting activities of bacterial endophytes isolated from the medicinal plant Pairs polyphylla var. yunnanensis. *World J Microbiol Biotechnol* 2021, *38*, 15, doi:10.1007/s11274-021-03194-0.
57. Mathur, P.; Chaturvedi, P.; Sharma, C.; Bhatnagar, P. Improved seed germination and plant growth mediated by compounds synthesized by endophytic Aspergillus niger (isolate 29) isolated from Albizia lebbeck (L.) Benth. *3 Biotech* 2022, *12*, 271, doi:10.1007/s13205-022-03332-x.
58. Purushotham, N.; Jones, E.; Monk, J.; Ridgway, H. Community Structure of Endophytic Actinobacteria in a New Zealand Native Medicinal Plant Pseudowintera colorata (Horopito) and Their Influence on Plant Growth. *Microb Ecol* 2018, *76*, 729-740, doi:10.1007/s00248-018-1153-9.
59. Khare, E.; Mishra, J.; Arora, N.K. Multifaceted Interactions Between Endophytes and Plant: Developments and Prospects. *Frontiers in Microbiology* 2018, *9*, doi:ARTN 2732
10.3389/fmicb.2018.02732.
60. Jia, M.; Chen, L.; Xin, H.L.; Zheng, C.J.; Rahman, K.; Han, T.; Qin, L.P. A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. *Frontiers in Microbiology* 2016, *7*, doi:ARTN 906
10.3389/fmicb.2016.00906.
61. Mili, C. Bioprospecting of endophytes associated with Solanum species: a mini review. *Arch Microbiol* 2023, *205*, 254, doi:10.1007/s00203-023-03596-8.
62. Li, X.; Lang, D.; Wang, J.; Zhang, W.; Zhang, X. Plant-beneficial Streptomyces dioscori SF1 potential biocontrol and plant growth promotion in saline soil within the arid and semi-arid areas. *Environ Sci Pollut Res Int* 2023, *30*, 70194-70212, doi:10.1007/s11356-023-27362-x.
63. Boonmahome, P.; Namwongsa, J.; Vorasoot, N.; Jogloy, S.; Riddech, N.; Boonlue, S.; Mongkolthanaruk, W. Single and co-inoculum of endophytic bacteria promote growth and yield of Jerusalem artichoke through upregulation of plant genes under drought stress. *PLoS One* 2023, *18*, e0286625, doi:10.1371/journal.pone.0286625.
64. Li, J.; Wu, H.; Pu, Q.; Zhang, C.; Chen, Y.; Lin, Z.; Hu, X.; Li, O. Complete genome of Sphingomonas paucimobilis ZJSH1, an endophytic bacterium from Dendrobium officinale with stress resistance and growth promotion potential. *Arch Microbiol* 2023, *205*, 132, doi:10.1007/s00203-023-03459-2.
65. Li, M.; Ren, Y.; He, C.; Yao, J.; Wei, M.; He, X. Complementary Effects of Dark Septate Endophytes and Trichoderma Strains on Growth and Active Ingredient Accumulation of Astragalus mongholicus under Drought Stress. *J Fungi (Basel)* 2022, *8*, doi:10.3390/jof8090920.

66. Wang, J.F.; Hou, W.P.; Christensen, M.J.; Li, X.Z.; Xia, C.; Li, C.J.; Nan, Z.B. Role of Epichloe Endophytes in Improving Host Grass Resistance Ability and Soil Properties. *J Agr Food Chem* 2020, *68*, 6944-6955, doi:10.1021/acs.jafc.0c01396.
67. Wu, W.; Chen, W.H.; Liu, S.Y.; Wu, J.J.; Zhu, Y.T.; Qin, L.P.; Zhu, B. Beneficial Relationships Between Endophytic Bacteria and Medicinal Plants. *Frontiers in Plant Science* 2021, *12*, doi:ARTN 646146 10.3389/fpls.2021.646146.
68. Godara, H.; Ramakrishna, W. Endophytes as nature's gift to plants to combat abiotic stresses. *Letters in Applied Microbiology* 2023, *76*, doi:10.1093/lambio/ovac067.
69. Chen, H.; Chen, J.; Qi, Y.; Chu, S.; Ma, Y.; Xu, L.; Lv, S.; Zhang, H.; Yang, D.; Zhu, Y.; et al. Endophytic fungus Cladosporium tenuissimum DF11, an efficient inducer of tanshinone biosynthesis in Salvia miltiorrhiza roots. *Phytochemistry* 2022, *194*, 113021, doi:10.1016/j.phytochem.2021.113021.
70. Xu, W.; Jin, X.; Yang, M.; Xue, S.; Luo, L.; Cao, X.; Zhang, C.; Qiao, S.; Zhang, C.; Li, J.; et al. Primary and secondary metabolites produced in Salvia miltiorrhiza hairy roots by an endophytic fungal elicitor from Mucor fragilis. *Plant Physiol Biochem* 2021, *160*, 404-412, doi:10.1016/j.plaphy.2021.01.023.
71. Masoudi Khorasani, F.; Ganjeali, A.; Asili, J.; Cheniany, M. Beneficial effects of endophytic fungi inoculation on tanshinones and phenolic compounds of Salvia abrotanoides. *Iran J Basic Med Sci* 2023, *26*, 408-413, doi:10.22038/IJBMS.2023.67730.14828.
72. Ye, H.T.; Luo, S.Q.; Yang, Z.N.; Wang, Y.S.; Ding, Q.; Wang, K.F.; Yang, S.X.; Wang, Y. Endophytic fungi stimulate the concentration of medicinal secondary metabolites in houttuynia cordata thunb. *Plant Signal Behav* 2021, *16*, 1929731, doi:10.1080/15592324.2021.1929731.
73. Xie, X.G.; Zhang, Z.Z.; Chen, L.; Ming, Q.L.; Sheng, K.X.; Chen, X.; Rahman, K.; Feng, K.M.; Su, J.; Han, T. An endophytic fungus Schizophyllum commune isolated from Panax ginseng enhances hairy roots growth and ginsenoside biosynthesis. *Can J Microbiol* 2023, doi:10.1139/cjm-2022-0194.
74. Palanichamy, P.; Krishnamoorthy, G.; Kannan, S.; Marudhamuthu, M. Bioactive potential of secondary metabolites derived from medicinal plant endophytes. *Egyptian Journal of Basic and Applied Sciences* 2019, *5*, 303-312, doi:10.1016/j.ejbas.2018.07.002.
75. Santra, H.K.; Banerjee, D. Antifungal activity of volatile and non-volatile metabolites of endophytes of Chloranthus elatior Sw. *Front Plant Sci* 2023, *14*, 1156323, doi:10.3389/fpls.2023.1156323.
76. Ahmad, T.; Bashir, A.; Farooq, S.; Riyaz-Ul-Hassan, S. Burkholderia gladioli E39CS3, an endophyte of Crocus sativus Linn., induces host resistance against corm-rot caused by Fusarium oxysporum. *J Appl Microbiol* 2022, *132*, 495-508, doi:10.1111/jam.15190.
77. Anand, U.; Pal, T.; Yadav, N.; Singh, V.K.; Tripathi, V.; Choudhary, K.K.; Shukla, A.K.; Sunita, K.; Kumar, A.; Bontempi, E.; et al. Current Scenario and Future Prospects of Endophytic Microbes: Promising Candidates for Abiotic and Biotic Stress Management for Agricultural and Environmental Sustainability. *Microb Ecol* 2023, doi:10.1007/s00248-023-02190-1.
78. Rajendran, S.; Robertson, L.P.; Kosgahakumbura, L.; Fernando, C.; Goransson, U.; Wang, H.; Hettiarachchi, C.; Gunasekera, S. Antibacterial eremophilane sesquiterpenoids from Xylaria feejeensis, an endophytic fungi of the medicinal plant Geophila repens. *Fitoterapia* 2023, *167*, 105496, doi:10.1016/j.fitote.2023.105496.
79. Cheng, Y.; Liu, G.; Li, Z.; Zhou, Y.; Gao, N. Screening saikosaponin d (SSd)-producing endophytic fungi from Bupleurum scorzonerifolium Willd. *World J Microbiol Biotechnol* 2022, *38*, 242, doi:10.1007/s11274-022-03434-x.
80. Yu, J.; Wang, J.P.; Liu, S.F.; Yin, C.Y.; Tang, D.Y.; Li, Y.H.; Zhang, L.X. 7-Methoxy-13-dehydroxypaxilline: New indole diterpenoid from an endophytic fungus Penicillium sp. Nb 19. *Nat Prod Res* 2022, 1-9, doi:10.1080/14786419.2022.2107639.
81. Gu, H.; Zhang, S.; Liu, L.; Yang, Z.; Zhao, F.; Tian, Y. Antimicrobial Potential of Endophytic Fungi From Artemisia argyi and Bioactive Metabolites From Diaporthe sp. AC1. *Front Microbiol* 2022, *13*, 908836, doi:10.3389/fmicb.2022.908836.
82. Santra, H.K.; Maity, S.; Banerjee, D. Production of Bioactive Compounds with Broad Spectrum Bactericidal Action, Bio-Film Inhibition and Antilarval Potential by the Secondary Metabolites of the Endophytic Fungus Cochliobolus sp. APS1 Isolated from the Indian Medicinal Herb Andrographis paniculata. *Molecules* 2022, *27*, doi:10.3390/molecules27051459.
83. Yehia, R.S. Multi-Function of a New Bioactive Secondary Metabolite Derived from Endophytic Fungus Colletotrichum acutatum of Angelica sinensis. *J Microbiol Biotechnol* 2023, *33*, 1-17, doi:10.4014/jmb.2206.06010.
84. Zhu, J.; Wang, Z.; Song, L.; Fu, W.; Liu, L. Anti-Alzheimer's Natural Products Derived from Plant Endophytic Fungi. *Molecules* 2023, *28*, doi:10.3390/molecules28052259.
85. Ahmed, A.M.; Mahmoud, B.K.; Millan-Aguinaga, N.; Abdelmohsen, U.R.; Fouad, M.A. The endophytic Fusarium strains: a treasure trove of natural products. *RSC Adv* 2023, *13*, 1339-1369, doi:10.1039/d2ra04126j.
86. Wang, Z.C.; Wang, L.; Pan, Y.P.; Zheng, X.X.; Liang, X.N.; Sheng, L.L.; Zhang, D.; Sun, Q.; Wang, Q. Research advances on endophytic fungi and their bioactive metabolites. *Bioproc Biosyst Eng* 2023, *46*, 165-170, doi:10.1007/s00449-022-02840-7.

87. Nzimande, B.; Makhwitine, J.P.; Mkhwanazi, N.P.; Ndlovu, S.I. Developments in Exploring Fungal Secondary Metabolites as Antiviral Compounds and Advances in HIV-1 Inhibitor Screening Assays. *Viruses* 2023, *15*, doi:10.3390/v15051039.
88. Xu, F.; Wang, S.; Li, Y.; Zheng, M.; Xi, X.; Cao, H.; Cui, X.; Guo, H.; Han, C. Yield enhancement strategies of rare pharmaceutical metabolites from endophytes. *Biotechnol Lett* 2018, *40*, 797-807, doi:10.1007/s10529-018-2531-6.
89. Mursyidah, A.K.; Hafizzudin-Fedeli, M.; Muhammad, N.A.N.; Latiff, A.; Firdaus-Raih, M.; Wan, K.L. Dissecting the Biology of Rafflesia Species: Current Progress and Future Directions Made Possible with High-Throughput Sequencing Data. *Plant Cell Physiol* 2023, doi:10.1093/pcp/pcad004.
90. Riva, V.; Mapelli, F.; Bagnasco, A.; Mengoni, A.; Borin, S. A Meta-Analysis Approach to Defining the Culturable Core of Plant Endophytic Bacterial Communities. *Appl Environ Microbiol* 2022, *88*, e0253721, doi:10.1128/aem.02537-21.
91. Chen, P.; Zhang, C.M.; Ju, X.Y.; Xiong, Y.W.; Xing, K.; Qin, S. Community Composition and Metabolic Potential of Endophytic Actinobacteria From Coastal Salt Marsh Plants in Jiangsu, China. *Frontiers in Microbiology* 2019, *10*, doi:ARTN 1063 10.3389/fmicb.2019.01063.
92. Pan, W.J.; Miao, L.Y.; Fan, S.P.; Lv, P.W.; Lin, A.H.; Geng, H.; Song, F.J.; Zhang, P. New insights into the composition and diversity of endophytic bacteria in cultivated Huperzia serrata. *Can J Microbiol* 2023, doi:10.1139/cjm-2022-0171.
93. Fitzgerald, M.; Heinrich, M.; Booker, A. Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. *Frontiers in Pharmacology* 2020, *10*, doi:ARTN 1480
10.3389/fphar.2019.01480.
94. Applequist, W.L.; Brinckmann, J.A.; Cunningham, A.B.; Hart, R.E.; Heinrich, M.; Katerere, D.R.; van Andel, T. Scientists & apos; Warning on Climate Change and Medicinal Plants. *Planta Med* 2020, *86*, 10-18, doi:10.1055/a-1041-3406.
95. Cheng, Q.Q.; Ouyang, Y.; Tang, Z.Y.; Lao, C.C.; Zhang, Y.Y.; Cheng, C.S.; Zhou, H. Review on the Development and Applications of Medicinal Plant Genomes. *Frontiers in Plant Science* 2021, *12*, doi:ARTN 791219
10.3389/fpls.2021.791219.
96. Hilario, S.; Goncalves, M.F.M. Endophytic Diaporthe as Promising Leads for the Development of Biopesticides and Biofertilizers for a Sustainable Agriculture. *Microorganisms* 2022, *10*, doi:ARTN 2453
10.3390/microorganisms10122453.
97. Dwibedi, V.; Rath, S.K.; Joshi, M.; Kaur, R.; Kaur, G.; Singh, D.; Kaur, G.; Kaur, S. Microbial endophytes: application towards sustainable agriculture and food security. *Appl Microbiol Biot* 2022, *106*, 5359-5384, doi:ARTN s00253-022-12078-810.1007/s00253-022-12078-8.
98. Singh, D.; Thapa, S.; Mahawar, H.; Kumar, D.; Geat, N.; Singh, S.K. Prospecting potential of endophytes for modulation of biosynthesis of therapeutic bioactive secondary metabolites and plant growth promotion of medicinal and aromatic plants. *Anton Leeuw Int J G* 2022, *115*, 699-730, doi:10.1007/s10482-022-01736-6.
99. Xia, Y.D.; Liu, J.N.; Chen, C.; Mo, X.L.; Tan, Q.; He, Y.; Wang, Z.K.; Yin, J.; Zhou, G.Y. The Multifunctions and Future Prospects of Endophytes and Their Metabolites in Plant Disease Management. *Microorganisms* 2022, *10*, doi:ARTN 1072
10.3390/microorganisms10051072.
100. Sharma, H.; Rai, A.K.; Dahiya, D.; Chettri, R.; Nigam, P.S. Exploring endophytes for in vitro synthesis of bioactive compounds similar to metabolites produced in vivo by host plants. *AIMS Microbiol* 2021, *7*, 175-199, doi:10.3934/microbiol.2021012.
101. Mandal, S.; Anand, U.; Lopez-Bucio, J.; Radha; Kumar, M.; Lal, M.K.; Tiwari, R.K.; Dey, A. Biostimulants and environmental stress mitigation in crops: A novel and emerging approach for agricultural sustainability under climate change. *Environ Res* 2023, 116357, doi:10.1016/j.envres.2023.116357.
102. Fontana, D.C.; de Paula, S.; Torres, A.G.; de Souza, V.H.M.; Pascholati, S.F.; Schmidt, D.; Neto, D.D. Endophytic Fungi: Biological Control and Induced Resistance to Phytopathogens and Abiotic Stresses. *Pathogens* 2021, *10*, doi:ARTN 570
10.3390/pathogens10050570.
103. Anand, K.; Kumar, V.; Prasher, I.B.; Sethi, M.; Raj, H.; Ranjan, H.; Chand, S.; Pandey, G.K. Bioactive molecules from fungal endophytes and their applications in pharmaceutical industries: challenges and future scope. *J Basic Microb* 2023, doi:10.1002/jobm.202200696.