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One New Host Records of *Apiospora arundinis* From *Lactarius vividus*

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Abstract

In this investigation, morphological traits and phylogenetic analyses of multiple genes, including ITS, 28S, tub2, and tef1, were employed to classify a companion fungus extracted from the fresh fruiting bodies of *Lactarius vividus* in China. The fungus was identified as *Apiospora arundinis*, marking the first instance of its isolation from a mushroom. Subsequent research into its biological characteristics revealed that the mycelium was the optimal condition of 30 ^{o}C, pH7 and grew well in medium containing soluble starch and peptidic sugars. It was also observed that this fungus was resent in the fruiting bodies of *Lactarius vividus*, and its hyphal growth was influenced by temperature, pH, and media composition. These findings lay a foundation for additional research and practical applications of *Apiospora arundinis*, which are of great significance for the storage and preservation of *Lactarius vividus*, guiding the cultivation of mycorrhizal seedlings, and exploring the mechanism and mechanism, but the specific principles need to be further explored.

Keywords: endophytic fungi, cultural conditions, mycelial growth

1. Introduction

Organisms do not exist in isolation in nature, but in complex network relationships. This network relationship includes food chain, symbiotic relationship, competitive relationship, parasitic relationship and more. These intricate relationships form an ecosystem wherein organisms become interdependent and engage in mutual interactions. The complex network of these relationships fosters interdependence among organisms and exerts mutual influence, collectively ensuring the equilibrium and stability of the ecosystem. Endophytes are microorganisms that reside within the tissues or organs and intercellular spaces of healthy hosts throughout various stages of their life cycle without inducing disease or, conversely, providing benefits to the host's growth and development. The majority of research indicates that endophytic fungi are prevalent in plants (Ahlholm et al., 2002), fungi (Vahdatzadeh et al., 2015), and animals (Wang et al., 2017a). The composition of these fungi varies significantly depending on geographical location, climatic conditions, habitat type, different tissues within the same host, and the host's various growth stages (Guo, 2001). Endophytes, a diverse group comprising bacteria, fungi, and actinomycetes, are often overlooked due to their asymptomatic presence within healthy plant tissues (Herre et al., 2007). These organisms inhabit a unique microenvironment within fruiting bodies, which offers the necessary conditions for the survival and propagation of specific endophytic species, including saprophytic and parasitic varieties (Wang et al., 2017c). Research has indicated that fruiting bodies harbor a rich endophytic bacterial community, a factor potentially contributing to the decay of edible fungi, yet also playing a crucial role in the life cycle of these fungi. Consequently, intensifying research on the diversity of Lactarius endophytes and the interplay between these endophytes and the growth and development of Lactarius fruiting bodies is pivotal for advancing our comprehension of Lactarius biology.

Endophytic fungi are not only species-rich but also capable of producing bioactive substances that are identical to, similar to, or even distinct from those of their hosts. They fulfill significant biological and ecological roles, including the regulation of host growth and development, insecticidal activity, bacterial inhibition, anti-tumor effects, and antiviral properties (Tan et al., 2001; Ma et al., 2004; Gunatilaka, 2006). Plant endophytes can secrete phytohormones or assist the host in absorbing additional nutrients, thereby fostering plant growth, enhancing plant resilience to stress, combating pests and diseases, and facilitating the accumulation of secondary

metabolites. This has substantial application value in agricultural production (Dubey et al., 2020). Animal endophytes have a long-term symbiotic relationship with their hosts, co-evolving and engaging in host metabolism, providing nutrients, and aiding in the degradation of harmful substances (Godfray, 2012; Thong-on et al., 2012; Ceja-Navarro et al., 2015). Similarly, endophytic bacteria are pivotal to the growth, development, health, and functionality of fungi. They can colonize fungal mycelia to promote mycelial growth, biomass increase, and fruiting body formation through metabolic complementation. They can also colonize fruiting bodies to occupy the ecological niches provided by the host and prevent the invasion of other bacteria or pathogens (Hildebrandt et al., 2002). For instance, research on endophytic bacteria in truffle fruiting bodies has primarily focused on these microorganisms, revealing that they contribute to the maturation and development of truffle fruiting bodies and the formation of aromatic compounds (Antonybabu et al., 2014; Splivallo et al., 2015).

Apiospora is a genus with a broad distribution. Xu et al. (2012) isolated *A. arundinis* from the soil of the Tropical Botanical Garden of the Chinese Academy of Sciences (Menglun) using dilution and soil particle methods. Chen et al. (2013) isolated the aroma-producing fungus A. montagnei from the surface of Lophatherum gracile leaves, and its volatile substances exhibited inhibitory effects on several plant pathogens, including Botrytis cinerea, Bipolaris oryzae, Fusarium graminearum, Alternaria brassicae and Sclerotinia sclerotiorum. Wang et al. (2020) isolated endophytic fungi A. montagnei and A. sphaerosperma , both of which possess strong tolerance to heavy metals, from Broussonetia papyrifera. Long et al. (2022) isolated strain *A. arundinis* from Phakellia fusca Thiele in the South China Sea (Xisha). *A. arundinis* is a multifunctional and significant endophytic fungus, capable of producing a range of secondary metabolites. It holds ecological value and is also of considerable medicinal importance (Zhu et al., 2011), as *A. arundinis* can synthesize alkaloids (Wang et al., 2015b; Zhang et al., 2019), benzochromones (Bao et al., 2018; Morishita et al., 2019), coumarins (Wang et al., 2017b; Tsukada et al., 2011), terpenoids (Ye et al., 2019), steroids (Elissawy et al., 2017), cytochalasins (Wang et al., 2017b) and xanthones (Wang et al., 2014), many of which exhibit potent antibacterial, antiviral, and cytotoxic activities. In summary, the investigation of endophytic diversity is crucial for understanding the interaction mechanisms between endophytes and their hosts, indicating the hosts' growth status and environmental conditions. It also aids in the development of biological resources with exceptional ecological and medicinal properties. This research will enable us to harness the potential of endophytes more effectively and foster the sustainable development of agriculture and ecology.

2. Materials and Methods

2.1 Morphological Studies

The strain was originally isolated from the fruit body of *Lactarius vividus*, and deposited in strains library of Guizhou Institute of Biology, GZCC0435. Descriptions of species are according to Pintos et al. (2021).

2.2 Phylogenetic Analyses

DNA extraction and PCR protocols followed Wang et al. (2015a). The following four regions were amplified: ITS, 28S, tub2 and tef1. Primer pairs ITS5(GGAAGTAAAAGTCGTAACAAGG) + ITS4(TCCTCCGCTTATTGATATGC), LROR(ACCCGCTGAACTTAAGC) + LR5(TCCTGAGGGAAACTTCG), 526F(GTCGTYGTYATYGGHCAYGT)+EF2(GGARGTACCAGTSATCATGTT) and T1(AACATGCGTGAGATTGTAAGT) + BT2B(ACCCTCAGTGTAGTGACCCTTGGC) were used to amplify the ITS, 28S, tub2 and tef1. The PCR products underwent purification and sequencing, conducted by Sangon Biotech in Shanghai, China.

2.3 Biological Characteristics Analysis

2.3.1 Single Factor Test of Carbon and Nitrogen Source

In the test of carbon source, 2% of fructose, white granulated sugar, maltose, soluble starch, yeast extract, beef powder, microcrystalline cellulose, trihydroxy aminomethane and citric acid were added into PDA medium to replace glucose.

For the nitrogen source test, 2% of peptone, soybean peptone, tryptone, yeast extract powder, urea, $NH_4H_2PO_4$, NH_4Cl, $(NH_4)_2SO_4$, $NaNO_3$, KNO_3 were added into PDA medium. No added served as a control.

Medium sterilization, mycelium inoculation and culture reference Shim et al. (2005). All experiments were performed in octuple. The measurement of mycelial growth was also conducted in accordance with the method outlined by Shim et al. (2005).

2.3.2 Single Factor Test of Inorganic Salt and Growth Factor

On the basis of PDA medium, 0.5% of ten inorganic salts ($CuSO_4$, $ZnSO_4$, $MnSO_4$, $MgSO_4$, $CaCl_2$, $FeSO_4$, $CdSO_4$) were added.

The growth factors of vitamins, including VB1, VB2, VB6, VB7, VB8, VB9, Leu, and Glu, were tested. Each was added at a concentration of 0.01 g/l to the basal PDA medium individually. No added as control.

2.3.3 Single Factor Test of Temperature and pH

A 4 mm diameter plug of an inoculum, selected from the edge of 5 days old cultures of *A. arundinis* grown on PDA with rubber puncher, was placed in the center of each agar plate of PDA. These medium were put to incubator with 15, 20, 25, 30 and 35 ^{o}C in the dark incubator, individually. pH natural.

To screen for a pH range favorable to the mycelial growth of *A. arundinis*, the basal medium PDA was adjusted to pH levels between 5 and 9 using 1 M NaOH or HCl, and subsequently incubated at 25 ^{o}C.

2.4 Data Processing

Excel software was used to sort out the data, and IBM SPSS Statistics 26.0 statistical software was used for data analysis.

3. Results

3.1 Taxonomy and Phylogenetic Analysis

Apiospora arundinis (Corda) (Pintos & Alvarado, 2021)

Description: Mycelial diameter 2-3.5 μm, smooth, transparent, branched, septate. Conidiophores degenerated into sporogenous cells. Conidiogenous cells 3-9 × 2-4 μm, clustered on the hyphae, smooth, spherical to hemispherical. Conidia 4-8 μm, globose, smooth. Sometimes sterile cells are mixed in the middle of conidia.

Culture characteristics: Colony flat, dense, medium aerial hyphae. The surface of the colony was dirty white with iron gray.

Notes: Phylogenetic analysis was conducted by amplifying and sequencing four genes: ITS, 28S, tub2, and tef1. Subsequent BLAST alignment analysis was performed in the NCBI database. The resulting gene sequences of *A. arundinis* were deposited in GenBank, under the following accession numbers: OR506148, OR506149, PP461302, PP320372. In the phylogenetic tree, the strains isolated in our study were clustered within the same branch as *A. arundinis*, and their morphological characteristics were found to be in agreement with those of the model strains (Figure 1). Consequently, this study confirmed the identification of these strains as *A. arundinis*. The strain is classified under Apiospora. Reference sequences were selected from the work of Pintos and Alvarado (2021) as indicated in Table 1, and a multi-gene phylogenetic tree (Figure 2) was constructed using Mega X software, employing the maximum likelihood method.

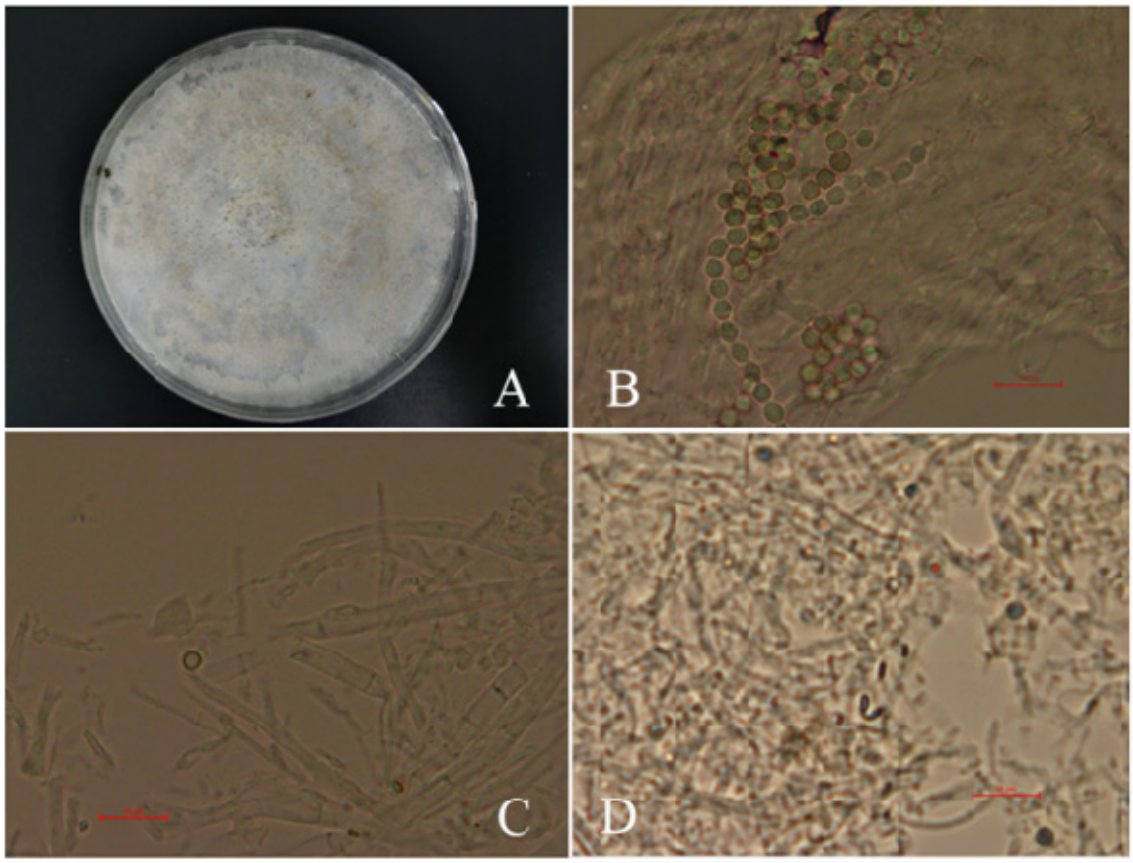

Figure 1. Culture traits and morphological characteristics of *A. arundinis*

Note. A. Colony on PDA. B. Conidiophores and conidia. C. Mycelial. D. Sterile cell.

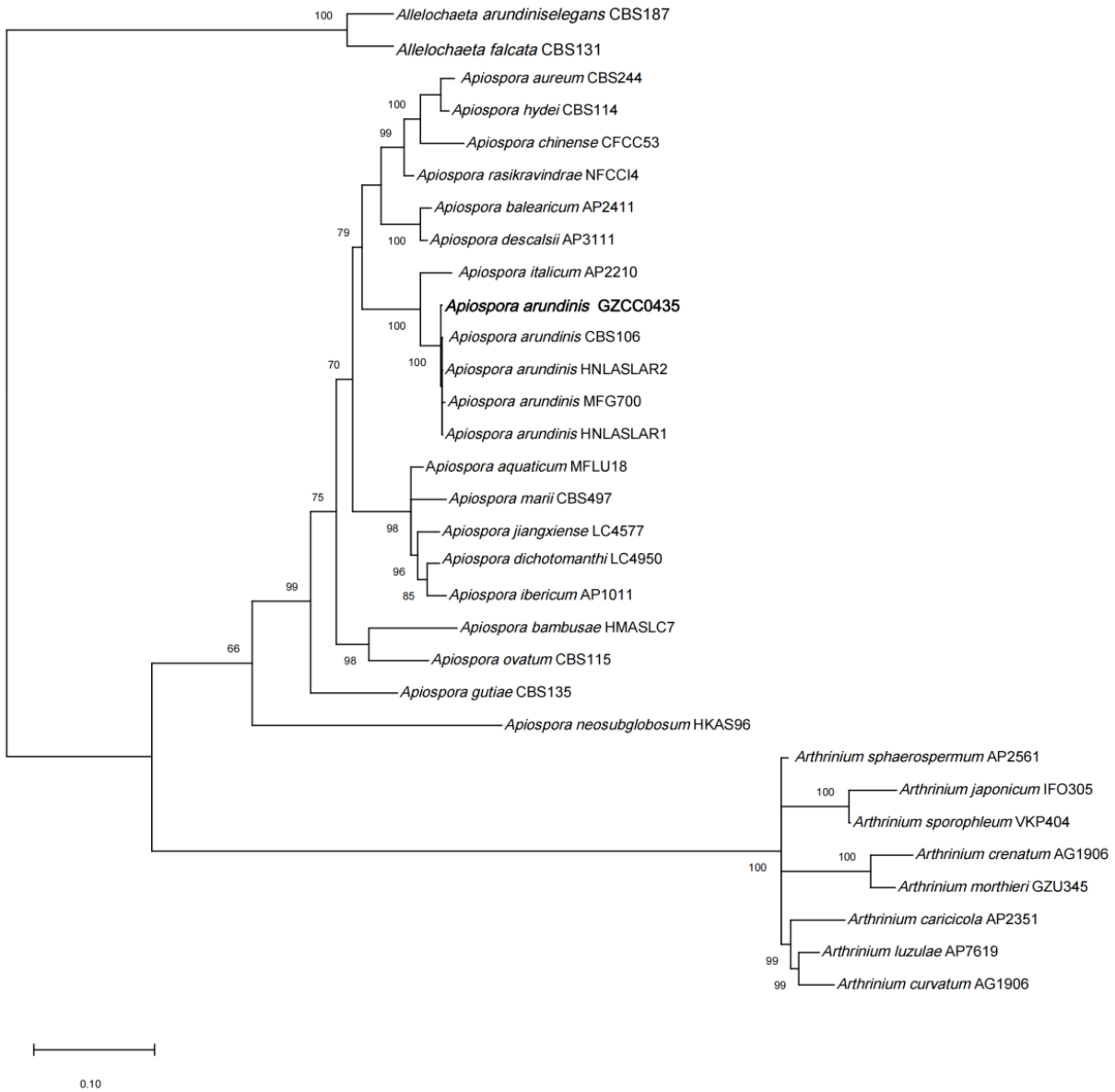

Figure 2. Maximum likelihood tree of *A. arundinis* based on ITS, 28S, tub2 and tef1

Table1. Details of strains included in the phylogenetic analyses

Species	Fungarium code	ITS	LSU	tef1	tub2
Allelochaeta elegans	CBS 187.81	MH554014	MH554234	MH554448	MH554690
Allelochaeta falcata	CBS 131117	MH553999	MH554217	MH554426	MH554668
Apiospora arundinis	MFG 70050	OK563249	-	OK626387	OK626371
Apiospora arundinis	HNLASL_AR2	MT664207	MT666066	MW768812	MW768814
Apiospora arundinis	HNLASL_AR1	MT664206	MT666065	MW768811	MW768813
Apiospora arundinis	CBS 106.12	KF144883	KF144927	KF145015	KF144973
Apiospora arundinis	**GZCC0435**	**OR506148**	**OR506149**	**PP320372**	**PP461302**
Apiospora aureum	CBS 244.83	MH861576	KF144935	KF145023	KF144981
Apiospora balearicum	AP24118, CBS 145129	MK014869	MK014836	-	MK017975
Apiospora bambusae	HMAS LC7106	KY494718	KY494794	KY806204	KY705186
Apiospora chinense	CFCC 53036	MK819291	-	MK818545	MK818547
Apiospora descalsii	AP31118A, CBS 145130	MK014870	MK014837	-	MK017976
Apiospora dichotomanthi	LC4950, CGMCC 3.18332	KY494697	KY494773	KY705096	KY705167
Apiospora gutiae	CBS 135835	KR011352	MH877577	KR011351	KR011350
Apiospora hydei	CBS 114990	KF144890	KF144936	KF145024	KF144982
Apiospora ibericum	AP10118, CBS 145137	MK014879	MK014846		MK017984
Apiospora italicum	AP221017, MA-Fungi 91733	MK014880	MK014847	MK017956	MK017985
Apiospora jiangxiense	LC4577, CGMCC 3.18381	KY494693	KY494769	KY705092	KY705163
Apiospora marii	CBS 497.90	MH873913	KF144947	KF145035	KF144993
Apiospora neosubglobosum	HKAS 96354	KY356090	KY356095	-	-
Apiospora ovatum	CBS 115042	KF144903	KF144950	KF145037	KF144995
Apiospora rasikravindrae	NFCCI:4158	MF461066	MF461172	-	-
Arthrinium aquaticum	MFLU 18-1628	MK828608	MK835806	-	-
Arthrinium caricicola	AP23518	MK014871	MK014838	MK017948	MK017977
Arthrinium crenatum	AG19066, CBS 146353	MW208931	MW208861	MW221917	MW221923
Arthrinium curvatum	AG19066, CBS 146353	MW208931	MW208861	MW221917	MW221923
Arthrinium japonicum	IFO 30500	AB220262	AB220356	-	AB220309
Arthrinium luzulae	AP7619-3	MW208937	MW208863	MW221919	MW221925
Arthrinium morthieri	GZU 345043	MW208938	MW208864	MW221920	MW221926
Arthrinium sphaerospermum	AP25619, CBS 146355	MW208943	MW208865	-	-
Arthrinium sporophleum	VK-P-4047/24	MW208946	MW208867	MW221922	MW221929

3.2 Effect of Carton Sources and Nitrogen on Mycelia Growth

To ascertain the carbon and nitrogen sources that best facilitate the mycelial growth of *A. arundinis*, ten different carbon and nitrogen sources were chosen to formulate the medium. The results indicated a significant impact of these sources on the mycelial growth (Figures 3 and 4). Notably, the medium utilizing soluble starch as the carbon source demonstrated the most rapid growth rate, reaching a mycelial growth of 9.58±0.51 mm/d. Conversely, media incorporating trihydroxy aminomethane or citric acid did not support growth. Among the nitrogen sources tested, peptone emerged as the most favorable, promoting the fastest growth rate of 9.58±0.51 mm/d. However, urea did not support mycelial growth.

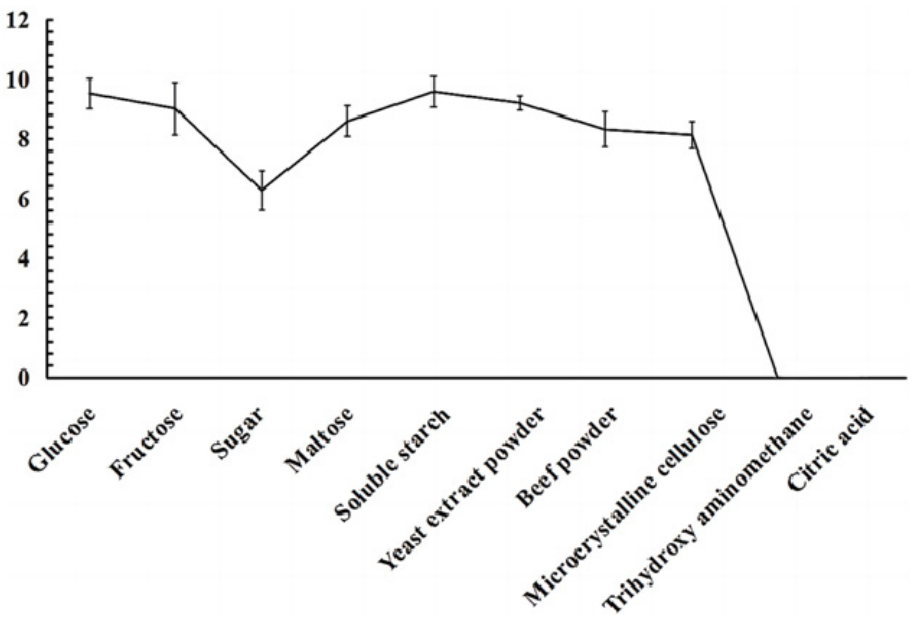

Figure 3. Effects of carbon sources on the mycelial growth of *A. arundinis*

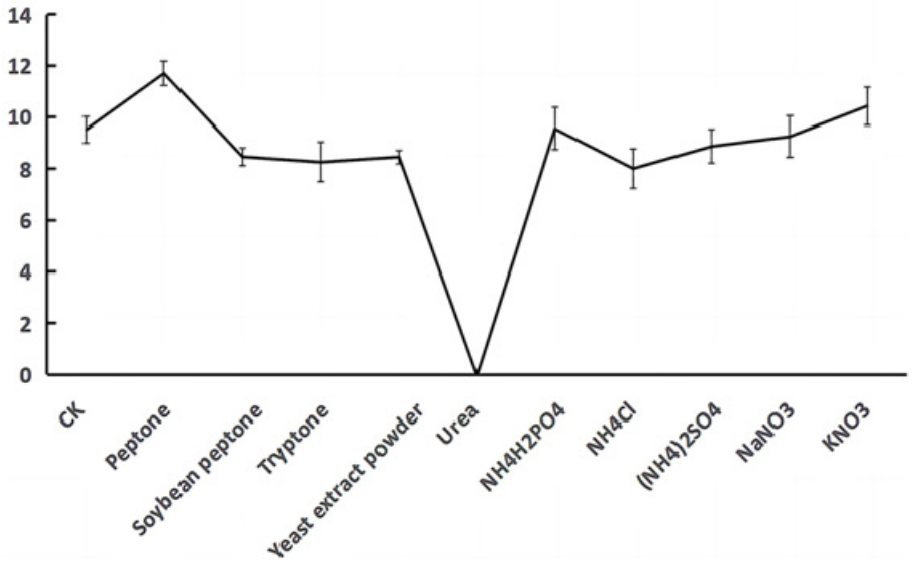

Figure 4. Effects of nitrogen sources on the mycelial growth of *A. arundinis*

3.3 Effect of Inorganic Salt and Growth Factor on Mycelial Growth

Among the 7 inorganic salts tested, with the exception of $CdSO_4$, which did not exhibit growth, the remaining salts were capable of promoting growth. However, only $MnSO_4$ and $CaCl_2$ surpassed the control (CK) in effectiveness (Figure 5). Others not only do not benefit, but also inhibit growth. For the growth factors test, the mycelium of *A. arundinis* can grow well by 8 growth factors (Figure 6). Specifically, only VB6 suppressed the mycelial growth of *A. arundinis*, while VB8 and Leu had no significant impact. The remaining factors outperformed the control. Consequently, we can choose to add two kinds of inorganic salts ($MnSO_4$, $CaCl_2$) and five kinds of growth factors (VB1, VB2, VB7, VB9, Glu) to promote mycelial growth.

Among the 7 inorganic salts tested, $CdSO_4$ was found to be non-supportive of growth, whereas the remaining salts exhibited growth-promoting capabilities. However, it is noteworthy that $MnSO_4$ and $CaCl_2$ demonstrated superior performance compared to the control (CK) in Figure 5. Conversely, certain inorganic salts not only failed to promote growth but also displayed inhibitory effects.

Regarding the growth factors evaluation, the mycelium of *A. arundinis* exhibited robust growth on 8 growth factors (Figure 6). Notably, VB6 was the only factor that inhibited the mycelial growth of *A. arundinis*. In contrast, VB8 and Leu did not exert significant effects, while the remaining growth factors demonstrated improved performance compared to the control.

Therefore, based on these findings, it is advisable to incorporate two types of inorganic salts ($MnSO_4$ and $CaCl_2$) along with five growth factors (VB1, VB2, VB7, VB9, and Glu) to effectively stimulate the mycelial growth of *A. arundinis*.

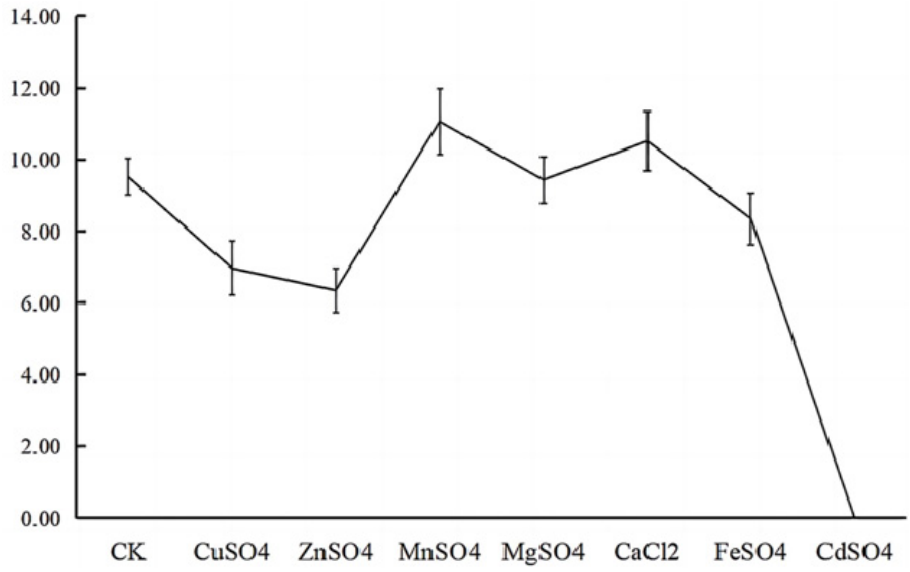

Figure 5. Effects of inorganic salt on the mycelial growth of *A. arundinis*

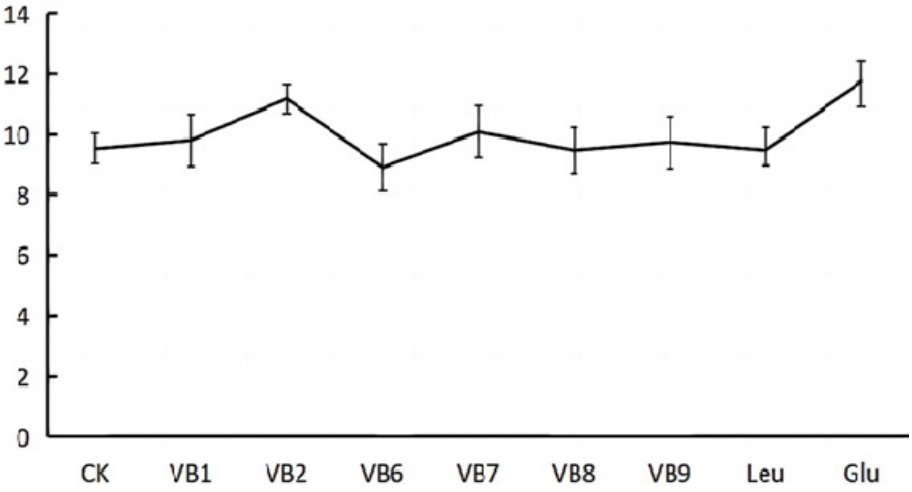

Figure 6. Effects of growth factor on the mycelial growth of *A. arundinis*

3.4 Effect of Temperature and pH on Mycelial Growth

The mycelium of *A. arundinis* is capable of proliferating within a temperature range of 15 to 35 ^{o}C, exhibiting notable variations in growth rates across various temperatures, as depicted in Figure 7. Remarkably, at 30 ^{o}C, the mycelium proliferates most rapidly, appearing white and dense, indicative of robust growth potential. Consequently, 30 ^{o}C is deemed as the optimal temperature for the mycelial growth of *A. arundinis*. Nevertheless, although mycelial growth progressively intensifies with increasing temperatures and reaches an optimal state at 30 ^{o}C, a temperature of 35 ^{o}C induces a marked slowdown and suppression of the mycelial growth rate.

The optimal pH range for fostering robust growth of *A. arundinis* has been determined to span from pH 5 to 9 (Figure 8). At pH 7, the mycelium exhibits the fastest growth rate, achieving superior growth conditions with thicker mycelium. However, at pH 8, the growth rate of the mycelium slows down significantly. In the case of *A. arundinis*, it is observed that growth under alkaline conditions is more favorable compared to acidic conditions.

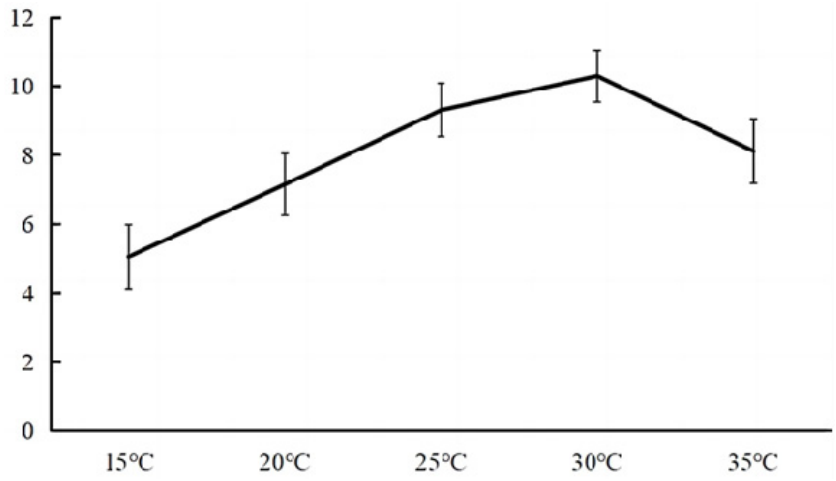

Figure 7. Effects of temperature on the mycelial growth of *A. arundinis*

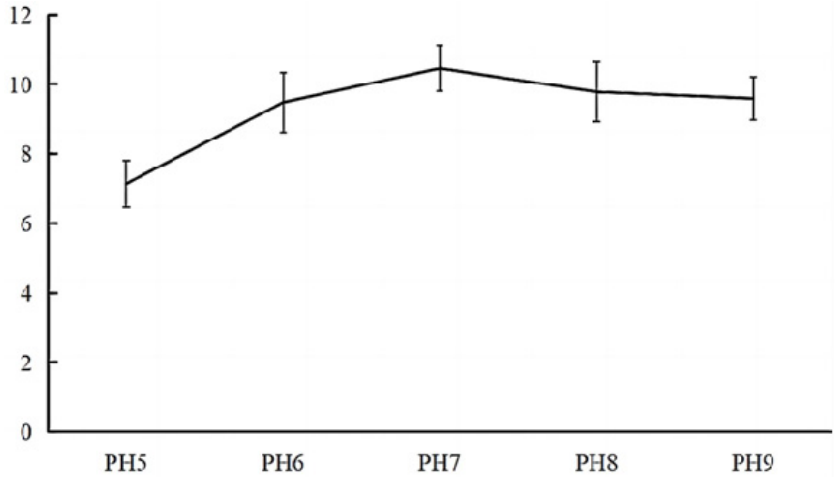

Figure 8. Effects of pH on the mycelial growth of *A. arundinis*

4. Discussion

GZCC0435 (*A. arundinis*) is an endophytic fungus isolated from the fruiting bodies of fresh and bright wild *Lactarius vividus*. Through the study of the biological characteristics of *A. arundinis*, we was found that it could grow well under a wide range of temperature, pH, and a variety of carbon and nitrogen sources, indicating its extensive environmental adaptability in practical applications (Kim et al., 2011; Crous & Groenewald, 2013). The optimum growth conditions of the strain were 30 ^{o}C, pH 7, and soluble starch peptone, consistent with the report of Zhang et al. (2021) and Gao et al. (2015). However, certain carbon sources, such as Trihydroxy aminomethane and Citric acid, nitrogen sources, including Urea, and inorganic salts like $CdSO_4$, can significantly impede mycelial germination. To expedite the production cycle and enhance the quality of GZCC0435, inorganic salts such as $MnSO_4$ and factors like Glu can be incorporated into the growth medium to foster mycelial proliferation.

This study marks the inaugural isolation of the endophytic fungus *A. arundinis* from the fresh fruiting bodies of *L. vividus*. Endophytes play an important role in the host micro-ecosystem. Based on long-term co-evolution,

endophytes and hosts have formed a mutually beneficial relationship. Endophytes can produce a variety of active substances, which have an important impact on the growth and development of the host. Liu (2024) reported that *A. arundinis* had strong nematicidal activity. It is well known that *Pinus* is the most common symbiotic tree species of *Lactarius*. Therefore, understanding the diversity of endophytic fungi within *Lactarius vividus* is instrumental in comprehending the mechanisms and ecological roles these fungi play within their host. It provides a scientific basis for better utilization of beneficial fungi and control of harmful fungi, and lays a foundation for the establishment of effective methods to promote the healthy growth, prevention and treatment of diseases, preservation and processing of *L. vividus*. At the same time, the biological characteristics of *A. arundinis* were studied, which filled the gap in the study of biological characteristics of *A. arundinis*, provided a scientific basis for nutrient extraction and component analysis, and provided the possibility for the development and sustainable utilization of subsequent resources.

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Authors Contributions

Jing Wang was responsible for study design, revising and manuscript drafting. Zhong-Xuan Liu and Ming-Fa Pan were responsible for data collection. Yi-Hua Yang revised it. All authors read and approved the final manuscript. Authors contributed equally to the study.

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