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Growth and Characteristics of Two Different *Epichloë sinensis* Strains Under Different Cultures

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In the present study, two *Epichloë sinensis* endophyte strains isolated from different *Festuca sinensis* ecotypes were inoculated on potato dextrose agar (PDA) and potato dextrose broth (PDB) media with or without (control) exogenous additives. After 4 weeks of growth, the growth (colony diameter, hyphal diameter, and mycelial biomass) and other characteristics (pH and antioxidant capacity of culture filtrate, mycelial ion contents, and hormone contents) were measured. The results showed that the culture conditions had significant effects ($p < 0.05$) on the hyphal diameter, mycelial biomass, and hormone content of the two strains. The mycelial biomass of the two strains in PDB was significantly higher ($p < 0.05$) than that on PDA. Except for strain 1 with indole-3-acetic acid (IAA) treatment and strain 84F with control and VB_1 treatments, the hyphal diameter of the two strains in PDB under the other treatments was significantly higher ($p < 0.05$) than that on PDA. In most cases, the IAA, cytokinins (CTK), abscisic acid (ABA), and gibberlic acid (GA) contents in the mycelia on PDA of the two strains were significantly higher ($p < 0.05$) than those in PDB. The two *E. sinensis* strains exhibited significantly different performances ($p < 0.05$) under the five treatments. The indices, including colony diameter, mycelial biomass, scavenging ability of superoxide anion radicals and hydroxyl radicals, pH of culture filtrate, ion contents, hyphal diameter, and IAA, CTK, GA, and ABA contents were significantly different ($p < 0.05$) between the two strains, although the performance was inconsistent. Exogenous additives had significant effects ($p < 0.05$) on the performance of the two *E. sinensis* strains. Indole-3-acetic acid and VB_1 treatments significantly promoted ($p < 0.05$) the growth of the two strains on both PDA and PDB. Indole-3-acetic acid treatment also significantly increased the hyphal diameters of the two strains in PDB ($p < 0.05$). Indole-3-acetic acid and VB_1 treatments significantly reduced ($p < 0.05$) the antioxidant ability of these two strains in PDB. NaCl and $ZnCl_2$ treatments had significant inhibitory effects ($p < 0.05$) on fungal growth and promotion effects on the antioxidant ability of the two strains. The treatments also had significant effects ($p < 0.05$) on hyphal diameters and ion and hormone contents, although the effects varied with different indices.

Keywords: *Epichloë sinensis*, potato dextrose agar, potato dextrose broth, exogenous additives, growth, characteristics

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INTRODUCTION

The grass endophytes live asymptomatically and internally within host plant tissues without causing visible damage to the plant (Siegel et al., 1987). Fungal hyphae grow in intercellular spaces in mostly leaf sheaths, culms, and seeds, while strains of *Epichloë* can be transmitted either vertically *via* seeds or horizontally *via* spores in stromata (Faeth et al., 2010). Fungal endophytes exhibit a variety of interactions with the host plant (Hume et al., 2016). The grass provides fungal endophytes with nutrients and space for growth, and in turn, fungal endophytes confer many benefits to grass hosts, including persistence/fitness, resistance/deterrence to insects, drought and salinity tolerance, and resistance to nematodes and fungal pathogens (Gundel et al., 2013; Vázquez-de-Aldana et al., 2013; Xia et al., 2018; Song et al., 2015a). This is attributed to the production of abundant and diverse secondary metabolites, as observed in a host and pure culture (Song et al., 2015b). Also, a diverse array of secondary metabolites isolated from *Epichloë* endophytes and symbionts is a rich source for developing new pesticides and drugs. However, some toxic alkaloids produced through the symbiosis also impair animal performance, which has significant economic consequences for the pastoral agricultural sectors (Schardl et al., 2012, 2013).

At present, studies on the biology and physiology of endophytes *in vitro* are mainly focused on the effects of media, conditions including temperature and pH, and carbon and nitrogen sources. For example, several studies suggested that the optimal growth conditions for *E. coenophiala* are 25°C and pH 9, whereas the optimal growth conditions of *E. gansuensis* are 25°C and pH 7 (Li et al., 2008). Latch et al. (1984) found that 25°C is the optimal temperature for the growth of *Epichloë festucae* var. *lolii*, *Epichloë coenophialum*, and *Epichloë typhina*. The optimal growth conditions for *E. festucae* are 25°C and pH 7–9 or 5 (Li et al., 2008; Ma, 2009), and the optimal growth conditions for *Epichloë bromicola* are 25°C and pH 5.09–6.10 or 9 (Zhang, 2013; Chen et al., 2016). Several studies have selected optimal nutrition resources for endophyte growth. Mannitol and tryptone are optimal carbon and nitrogen sources for *E. coenophiala* (Kulkarni and Nielsen, 1986; Pope and Hill, 1991), and maltose and tryptone are optimal carbon and nitrogen sources for *E. bromicola* (Chen et al., 2016). Lactose and casein are the optimal carbon and nitrogen sources for *E. festucae* (Li et al., 2008). Ferguson et al. found that the best nitrogen sources for the mycelial growth of *E. coenophiala* are proline and potassium nitrate (Ferguson et al., 1993). The growth of *E. festucae* is significantly improved by cellulase, tryptone, casein peptone, proline, and peptone (Ma, 2009).

Festuca sinensis is native to the cool semi-arid regions of China and is an important perennial cool-season bunchgrass involved in grassland establishment and ecological management programs in the Qinghai-Tibetan plateau in China (Tian et al., 2015; Zhou et al., 2015a; Wang et al., 2016). It is frequently symbiotic with *Epichloë* endophytes (Nan, 1996a). This endophyte has been isolated and identified through morphology with colony, texture, conidia, conidiophores, and its phylogeny studied through housekeeping genes. These evaluations confirmed that the endophyte that establishes symbiosis with *F. sinensis* is the new species, *Epichloë sinensis* (Tian et al., 2020). This species needs further and comprehensive studies to fully understand its characteristics and provides more reference for utilization. Previous studies have indicated that *E. sinensis* isolated from different host ecotypes performed morphological diversity which had different growth rates (Wang, 2019). Jin et al. (2009) showed that *E. sinensis* grows at 10–30°C and stop growing at 5°C and 35°C. The optimal growth conditions for *E. sinensis* are 25°C and pH 7–9, and the optimal carbon and nitrogen sources are mannitol and yeast extract. The ability to use carbon and nitrogen nutrients is different for different endophyte strains. Wang (2019) found that plant growth regulators [PGRs; VB_1, VB_5, VB_9, Indole-3-acetic acid (IAA), gibberellin (GA_3), and KT-30] promote *E. sinensis* strain growth, whereas ions (Na^+, Cd^{2+}, Cr^{6+}, and Zn^{2+}) inhibit their growth although the effects depend on additives and concentrations. These studies have mainly focused on the growth of *E. sinensis* under different temperatures, pH, carbon, nitrogen sources, vitamins, and heavy metals. However, little is known about the biological and physiological characteristics of *E. sinensis* in different media.

Production of fungal mycelium with high levels of secondary metabolites is critical for endophyte utilization. Compared with PDB culture for mycelium production, potato dextrose agar (PDA) culture is usually used to production (You et al., 2012), but PDA medium is not well for short-term culture to produce fungal mycelium. In addition, biomass and the biological activity of the mycelium of *Epichloë* endophytes have never been compared as between PDA and PDB culture.

In the present study, we selected two *E. sinensis* endophyte strains (strain ID 1, 84F) which were isolated from different *F. sinensis* ecotypes distributed in Gansu Province (1) and Qinghai Province (84F), China. The biological and physiological characteristics of these two strains on PDA and in PDB with different additives were measured. The objectives of this study were to: (1) compare the biological and physiological characteristics of these two *E. sinensis* strains grown in various media with exogenous additives; (2) clarify the response mechanisms of these two strains to different additives; and (3) explore the optimal enrichment conditions for *E. sinensis* mycelia.

MATERIALS AND METHODS

Biological Materials

Epichloë sinensis strain 1 isolated from wild *F. sinensis* seeds in Xiahe County, Gansu Province, and strain 84F isolated from wild *F. sinensis* seeds in Ping'an County, Qinghai Province, were provided by our research groups (Kuang, 2016) and preserved in the Mycological Herbarium of Lanzhou University, China. The hyphae were transferred onto fresh PDA plates ($D = 90$ mm) and incubated in the dark at 25 ± 1°C for 4 weeks before the experiment.

Experimental Design

Exogenous additives, including Vitamin B_1 (VB_1), IAA, sodium chloride (NaCl), and zinc chloride ($ZnCl_2$), were dissolved in

sterile water to make 30 g/L, 20 g/L, 23376 g/L, and 750 g/L concentrated solutions; 10 μl or 100 μl of each concentrated solution was added to 1 L sterilized PDA or 100 ml sterilized PDB, respectively, to reach final concentrations of 30 mg/L, 20 mg/L, 0.4 mol/L, and 750 mg/L, respectively. potato dextrose agar and PDB without additives were prepared as controls. A 6-mm-diameter inoculum plug of each fungus was inoculated on cellophane-overlaid PDA ($D = 90$ mm) containing different additives and incubated in the dark at 25 ± 1°C for 4 weeks. Each fungus had four replicates per treatment. Three 6-mm-diameter inoculum plugs of each fungus were also inoculated into PDB (100 ml in 250 ml flask). Each fungus had four replicates per treatment. All flasks were incubated at 25 ± 1°C with 145 rpm for 4 weeks.

Experimental Evaluations

Colony Diameter on PDA

The colony diameter on PDA was measured weekly during the first 4 weeks of growth.

Hypha Diameter

Pieces of adhesive tape were gently pressed over actively growing margins of colonies on PDA to collect the mycelium. The tapes were aseptically placed on sterile glass slides for observation using an Olympus optical microscope (BX51 SZX12 type; Mm et al., 2012). Pieces of mycelia in PDB were placed on sterile glass slides and then gently covered with coverslips. Each hypha was measured through microscopy with at least 50 measurements to determine the average mycelial diameter.

Mycelial Biomass

Mycelia on PDA were harvested by gentle scraping with a scalpel. Mycelia and culture filtrate in PDB were separated by centrifugation at 4°C and 8,000 rpm for 5 min. The culture filtrate was stored at −20°C until further use. The mycelia were washed thoroughly with sterile water, and sterile water was drained off with sterilized filter paper. Each harvested mycelium was transferred into an Eppendorf tube and weighed.

The pH Value of Culture Filtrate

A pH meter (PB-20 type) was used to measure the pH value of the culture filtrate.

Antioxidant Activity of Culture Filtrate

The culture filtrate was centrifuged at 10,000 rpm for 10 min, and the supernatant was collected to measure the total antioxidant capacity (T-AOC). Total antioxidant capacity was determined with an ultraviolet-visible spectrophotometer according to the procedure of the Total Antioxidant Capacity Assay Kit (Nanjing Jiancheng Company, China). The absorbance of the mixture was measured at 520 nm (Zeng et al., 2015).

The culture filtrate was evaporated under reduced pressure (8 × 103 Pa) and extracted twice with 75% ethyl alcohol. Subsequently, the extract was centrifuged at 5,000 rpm for 10 min. The supernatant was evaporated under reduced pressure to obtain the final extract, which was stored at 4°C for further testing.

The scavenging ability of superoxide anion radicals and hydroxyl radicals was determined through the pyrogallol autoxidation and salicylic acid methods, respectively (Kim et al., 1995; Škerget et al., 2005).

Ion Content of Mycelia

The mycelia were freeze-dried and ground to determine the ion content. Na^+, K^+, and Ca^{2+} ions were measured using atomic absorption spectrometry (M6AA system, Thermo, United States) after mineralization in a mixture of acids (HNO_4: $HClO_4$ = 4:1), and the Na^+/K^+ ratio was calculated (Hanway and Heidel, 1952).

Endogenous Hormone Content

After 28 days of growth, hyphae and culture filtrate were collected for GA_3, IAA, cytokinins (CTK), and abscisic acid (ABA) content determination using enzyme-linked immunosorbent assay (Danshi biology, Shanghai, China; Weiler, 1984). The percentage of hormone content in the culture filtrate in total content was calculated by dividing the hormone content in the culture filtrate by the total content secreted by *E. sinensis*.

Statistical Analyses

Statistical data analysis was performed with SPSS 25.0 (SPSS, Inc., Chicago, IL, United States). All averages and the standard error of the difference (*SE*) of the measurements were recorded in Excel2010. Univariate analysis of general linear models was employed to estimate the effects of single factor and their interaction on indices of *E. sinensis* strains in the present study (**Supplementary Tables S1, S2, S3**). Significant difference between single factor (exogenous additive, strain, and culture condition) was assessed by least significant difference tested at $p < 0.05$ and generated from one-way ANOVA based on the separated dataset. Statistical significance was defined at the 95% confidence level.

RESULTS

Colony Diameter

The colony diameters of the two *E. sinensis* strains were significantly different ($p < 0.05$), and the colony diameter of strain 1 was always significantly higher ($p < 0.05$) than that of strain 84F (**Table 1**).

Exogenous additives had significant effects ($p < 0.05$) on the growth of the two strains. Compared with control, IAA and VB_1 significantly promoted ($p < 0.05$) the growth of strains 1 and 84F, whereas NaCl and $ZnCl_2$ significantly inhibited ($p < 0.05$) the growth of the two strains (**Table 1**).

Hyphal Diameter

Culture conditions significantly affected the hyphal diameters of the two *E. sinensis* strains. The hyphal diameter of strains

TABLE 1 | Colony diameter of *Epichloë sinensis* under different treatments for 4 weeks (cm).

Treatment	Strain ID	
	1	84F
CK	2.93 ± 0.08c	1.95 ± 0.13c
Indole-3-acetic acid (IAA)	3.24 ± 0.08b	2.11 ± 0.07b
VB_1	3.52 ± 0.06a	2.41 ± 0.11a
NaCl	2.3 ± 0.12d	1.5 ± 0.08d
$ZnCl_2$	1.94 ± 0.07e	1.14 ± 0.16e
	*	

**in the table indicates significant differences between different strains ($p < 0.05$). Different lowercase letters in the table indicate significant differences between different exogenous additives ($p < 0.05$).*

in PDB was significantly higher ($p < 0.05$) than that on PDA (**Figure 1A**).

The hypha diameters of the two strains in PDB were significantly different ($p < 0.05$). The hyphal diameter of strain 1 was significantly lower ($p < 0.05$) than that of strain 84F (**Figure 1A**).

Exogenous additives had significant effects ($p < 0.05$) on hyphal diameters of the two strains. The hyphal diameters of strain 1 on PDA with control, VB_1, and NaCl treatments had no significant difference and were significantly lower ($p < 0.05$) than that with $ZnCl_2$ treatment. The hyphal diameters of strain 84F on PDA with control, IAA, and VB_1 treatments had no significant difference, which was significantly higher ($p < 0.05$) than that with NaCl and $ZnCl_2$ treatments. The hyphal diameters of strain 1 in PDB with IAA and $ZnCl_2$ were significantly higher ($p < 0.05$) than those with the other three treatments; the hyphal diameters of strain 84F in PDB were significantly different and were the highest with $ZnCl_2$ and the lowest with control and VB_1 (**Figure 1A**).

Mycelial Biomass

Culture conditions had significant effects ($p < 0.05$) on the mycelial biomass of the two *E. sinensis* strains. The mycelial biomass of the two strains in PDB was significantly higher ($p < 0.05$) than that on PDA (**Figure 1B**).

The mycelial biomass of the two strains in PDB was significantly different ($p < 0.05$). The mycelial biomass of strain 1 was significantly higher ($p < 0.05$) than that of strain 84F (**Figure 1B**).

Exogenous additives had significant effects ($p < 0.05$) on mycelial biomass of the two strains. On PDA, the mycelial biomass of strain 1 was significantly different ($p < 0.05$) under these five treatments and changed according to the following order depending on the additive: $VB_1 > IAA > CK > NaCl > ZnCl_2$. The mycelial biomass of strain 84F was also significantly different ($p < 0.05$) and changed according to the following order of additives: VB_1, $IAA > CK > NaCl > ZnCl_2$. In PDB with IAA and VB_1, the mycelial biomass of strain 1 was significantly higher ($p < 0.05$) than that with control treatment; however, in PDB with NaCl and $ZnCl_2$, biomass of strain 1 was significantly lower ($p < 0.05$) than that with control. In PDB with IAA, VB1, and control treatments, biomass of strain 84F were not significant difference ($p > 0.05$), which were significantly higher ($p < 0.05$) than those with NaCl and $ZnCl_2$ treatments (**Figure 1B**).

PH

Exogenous additives had significant effects ($p < 0.05$) on the pH of the culture filtrate of the two *E. sinensis* strains. The pH of strain 1 was the highest with NaCl treatment and was significantly higher ($p < 0.05$) than that with IAA, VB_1, and $ZnCl_2$ treatments. The pH of the filtrate of strain 84F with NaCl treatment was significantly higher ($p < 0.05$) than that with the other treatments, and the pH of strain 84F with VB_1 treatment was significantly lower ($p < 0.05$) than that with IAA, NaCl, and $ZnCl_2$ treatments (**Figure 2A**).

The Antioxidant Activity

Total Antioxidant Activity

Exogenous additives had significant effects ($p < 0.05$) on the T-AOC of the two *E. sinensis* strains. The T-AOC of strain 1 with control, NaCl, and $ZnCl_2$ treatments was significantly higher ($p < 0.05$) than that with IAA and VB_1 treatments. The T-AOC of strain 84F with NaCl and $ZnCl_2$ was significantly higher ($p < 0.05$) than that with IAA and VB_1 treatments (**Figure 2B**).

Superoxide Anion Radical and Hydroxyl Radical Scavenging Ability

The superoxide anion radical and hydroxyl radical scavenging abilities of the two *E. sinensis* strains were significantly different ($p < 0.05$). The superoxide anion radical and hydroxyl radical scavenging abilities of strain 84F were always significantly higher ($p < 0.05$) than that of strain 1 (**Figures 2C**,**D**).

Exogenous additives had significant effects ($p < 0.05$) on the superoxide anion radical and hydroxyl radical scavenging abilities of the two strains. The superoxide anion radical scavenging ability of strain 1 with $ZnCl_2$ treatment was significantly higher ($p < 0.05$) than that with control, IAA, and VB_1 treatments, and that with NaCl treatment was significantly higher ($p < 0.05$) than that with control and IAA treatments. The superoxide anion radical scavenging ability of strain 1 was the lowest with IAA treatment. The superoxide anion radical scavenging ability of strain 84F with IAA and VB_1 treatments was significantly lower ($p < 0.05$) than that with control, NaCl, and $ZnCl_2$ treatments. The hydroxyl radical scavenging ability of strains 1 and 84F with NaCl treatment was significantly higher ($p < 0.05$) than that with the other four treatments, while the scavenging ability with $ZnCl_2$ treatment was significantly higher ($p < 0.05$) than that with control, IAA, and VB_1 treatments (**Figures 2C**,**D**).

Ion Content of Mycelia

Exogenous additives had significant effects ($p < 0.05$) on the Na^+, K^+, and Ca^{2+} contents of the two *E. sinensis* strains. Na^+ and K^+ contents of strain 1 with IAA, VB_1, and $ZnCl_2$ were significantly higher ($p < 0.05$) than those with control and NaCl treatments. The Na^+ content of strain 84F with VB_1 treatment was significantly higher ($p < 0.05$) than that with control and

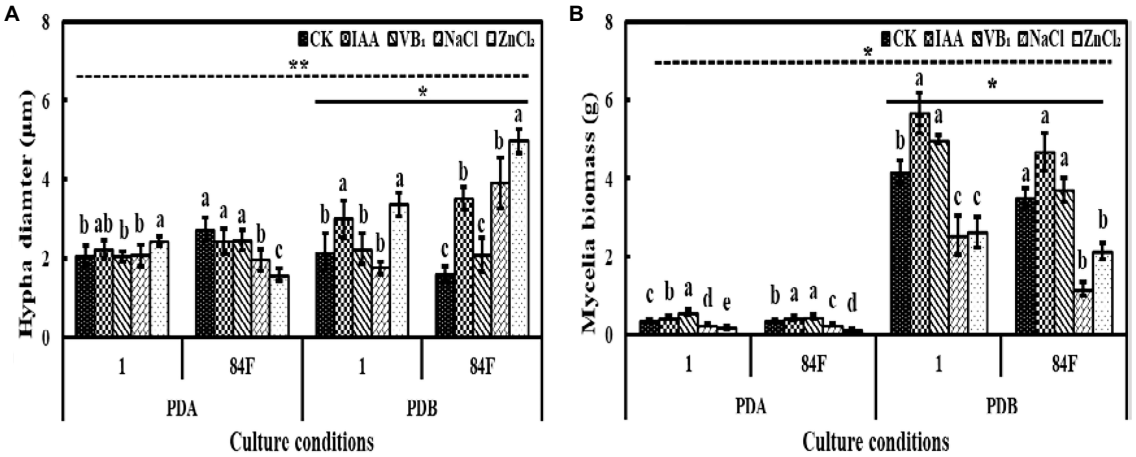

FIGURE 1 | Hyphal diameter and mycelial biomass of *Epichloë sinensis* under different treatments for 4 weeks. **(A)** is hyphal diameter, and **(B)** is mycelial biomass. Different lowercase letters in the figure indicate significant differences between different exogenous additives ($p < 0.05$); *on the real line in the figure indicates significant differences between different strains under same culture conditions ($p < 0.05$); *on the dotted line in the figure indicates significant differences between different cultures ($p < 0.05$).

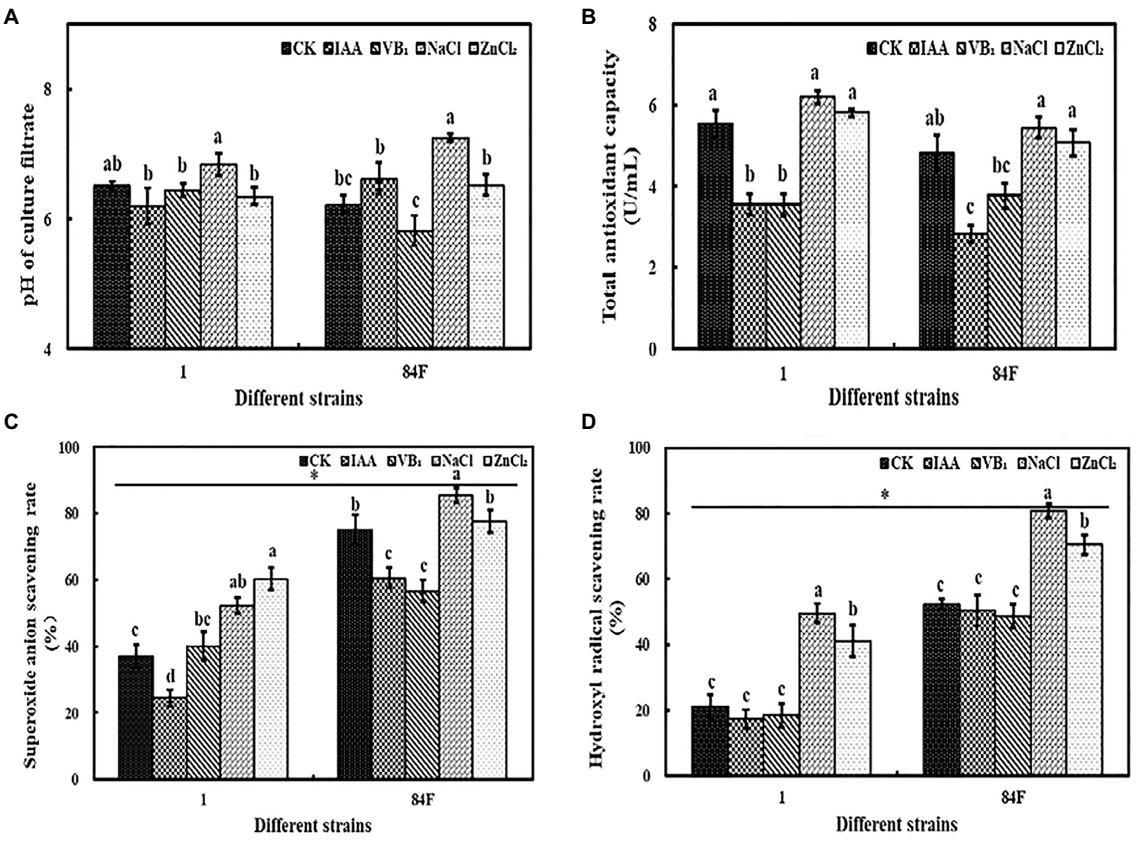

FIGURE 2 | PH and antioxidant ability of *E. sinensis* under different treatments for 4 weeks. **(A)** is PH, **(B)** is total antioxidant capacity of culture filtrate, **(C)** is superoxide anion radicals scavenging ability, and **(D)** is hydroxyl radical scavenging ability. The same as **Figure 1**.

NaCl treatments. The K^+ content of strain 84F with VB_1 treatment was significantly higher ($p < 0.05$) than that with IAA, NaCl, and $ZnCl_2$ treatments. The Ca^{2+} content of strain 1 with VB_1 treatment was significantly higher ($p < 0.05$) than that with control, NaCl, and $ZnCl_2$ treatments. The Ca^{2+} content of strain 84F with VB_1 treatment was significantly higher ($p < 0.05$) than

that with $ZnCl_2$ treatment. There was no significant difference between Na^+, K^+, and Ca^{2+} contents in mycelia of the two strains (**Figures 3A–C**).

Exogenous additives had significant effects on the Na^+/K^+ ratio of the two strains. Na^+/K^+ of strain 1 with NaCl treatment was significantly lower ($p < 0.05$) than that with control. Na^+/K^+ of strain 84F with control was significantly lower ($p < 0.05$) than that of IAA, VB_1, and $ZnCl_2$ treatments, and the ratio with IAA treatment was significantly higher ($p < 0.05$) than that with the other three treatments (**Figure 3D**).

Hormone Content

CTK Content

Culture conditions had significant effects ($p < 0.05$) on CTK content in mycelia of the two *E. sinensis* strains. The CTK content in mycelia of *E. sinensis* strains on PDA was significantly higher ($p < 0.05$) than that in PDB (**Figure 4**).

Cytokinins content in mycelia of the two strains on PDA and in PDB was not significantly different ($p < 0.05$). Cytokinins contents in the culture filtrate of strain 84F were significantly higher ($p < 0.05$) than that of strain 1 (**Figure 4**).

Exogenous additives had significant effects ($p < 0.05$) on CTK content on PDA and in PDB of the two strains. Cytokinins contents in mycelia of strain 1 on PDA and in PDB were not significantly different among the five treatments. The CTK contents of strain 84F on PDA with IAA and $ZnCl_2$ treatments were significantly higher ($p < 0.05$) than those with VB_1 treatment. In PDB with IAA, the CTK content in mycelia of strain 84F was significantly higher ($p < 0.05$) than that with NaCl treatment. Cytokinins content in the culture filtrate of strain 1 with IAA treatment was significantly higher ($p < 0.05$) than that with control and NaCl treatments; CTK content in the culture filtrate of strain 84F with control treatment was significantly higher ($p < 0.05$) than that with VB_1 treatment (**Figure 4**).

ABA Content

Culture conditions had significant effects ($p < 0.05$) on CTK content in mycelia of the two *E. sinensis* strains. Abscisic acid content in mycelia of *E. sinensis* strains on PDA was significantly higher ($p < 0.05$) than that in PDB (**Figure 5**).

The ABA content in mycelia of these two strains was significantly different ($p < 0.05$). In the culture filtrate, the ABA content of strain 84F was significantly higher ($p < 0.05$) than that of strain 1 (**Figure 5**).

Exogenous additives had significant effects ($p < 0.05$) on the ABA content of the two strains. On PDA, the ABA contents of strain 1 with control and $ZnCl_2$ treatments were significantly higher ($p < 0.05$) than those with NaCl treatment; the ABA

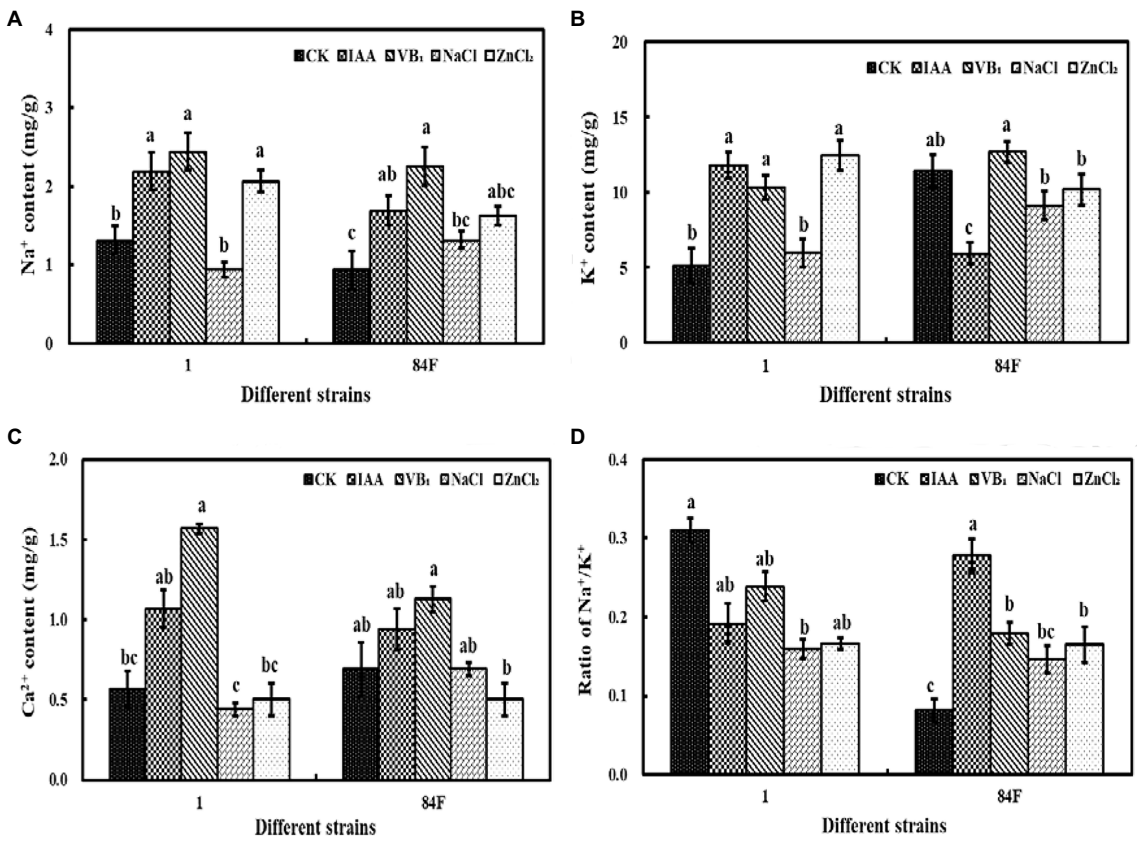

FIGURE 3 | Na^+, K^+, and Ca^{2+} contents and ratio of Na^+/K^+ of mycelium of *E. sinensis* under different treatments for 4 weeks. **(A)** is Na^+ content, **(B)** is K^+ content, **(C)** is Ca^{2+} content, and **(D)** is ratio of Na^+/K^+. The same as **Figure 1**.

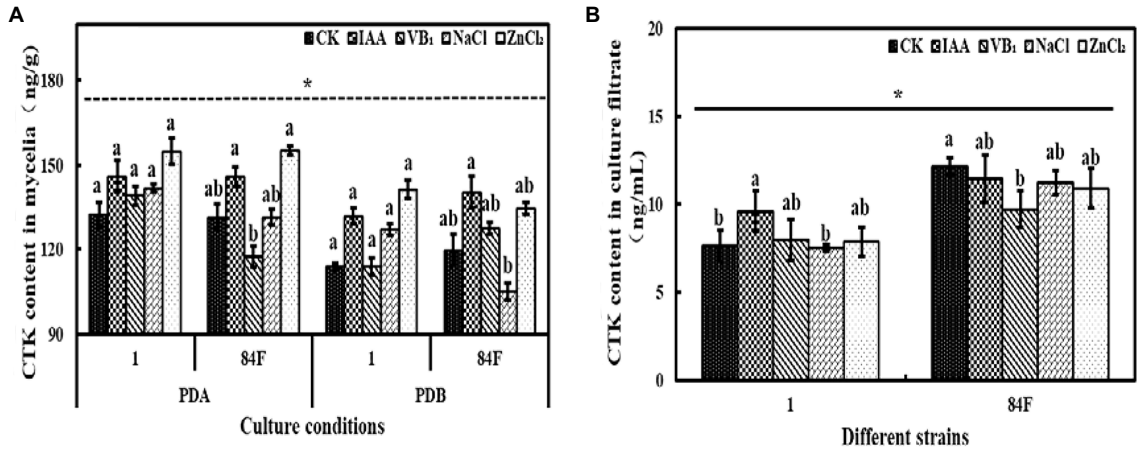

FIGURE 4 | Cytokinins content of *E. sinensis* under different treatments for 4 weeks. **(A)** is CTK content in mycelia, and **(B)** is CTK content in culture filtrate. The same as **Figure 1**.

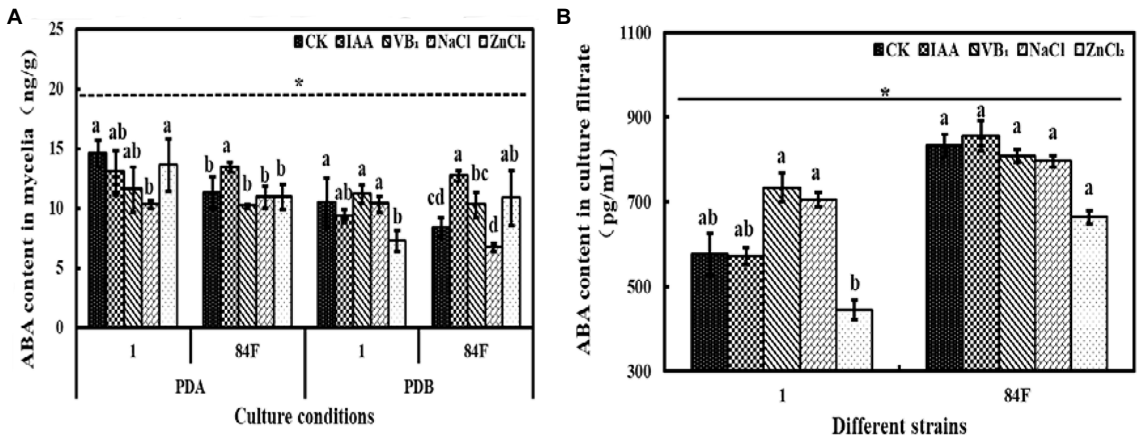

FIGURE 5 | Abscisic acid content of *E. sinensis* under different treatments for 4 weeks. **(A)** is ABA content in mycelia, and **(B)** is ABA content in culture filtrate. The same as **Figure 1**.

content for strain 84F with IAA treatment was significantly higher ($p < 0.05$) than that with the other four treatments. In PDB, ABA contents in the mycelia of strain 1 with control, VB_1, and NaCl treatments were significantly higher ($p < 0.05$) than those with $ZnCl_2$ treatment. Abscisic acid content in the mycelia of strain 84F with IAA treatment was significantly higher ($p < 0.05$) than that with control, VB_1, and NaCl treatments. In PDB with $ZnCl_2$ treatment, ABA content in mycelia of strain 84F was significantly higher ($p < 0.05$) than that with control and NaCl treatments. The ABA contents in the culture filtrate of strain 1 with VB_1 and NaCl treatments were significantly higher ($p < 0.05$) than those with $ZnCl_2$ treatment (**Figure 5**).

GA Content

Culture conditions had significant effects ($p < 0.05$) on GA content in mycelia of the two *E. sinensis* strains. GA content in mycelia of *E. sinensis* strains on PDA was significantly higher ($p < 0.05$) than that in PDB (**Figure 6**).

GA content in mycelia of the two strains was significantly different ($p < 0.05$). On PDA, the GA content of strain 1 was significantly higher ($p < 0.05$) than that of 84F. In PDB, no significant difference was observed between the two strains. In the culture filtrate, the GA content of strain 84F was significantly higher ($p < 0.05$) than that of strain 1 (**Figure 6**).

Exogenous additives had significant effects ($p < 0.05$) on the GA content of the two strains. On PDA, the GA content of strain 1 with control was significantly higher ($p < 0.05$) than that with VB_1 treatment. The GA content of strain 84F with IAA treatment was significantly higher ($p < 0.05$) than that with control, NaCl, and $ZnCl_2$ treatments. In PDB, there were no significant differences between GA contents in mycelia of strains 1 and 84F under the five treatments. GA content in the culture filtrate of strain 1 with IAA treatment was significantly lower ($p < 0.05$) than that with control, NaCl, and $ZnCl_2$ treatments; GA contents in the culture filtrate of strain 84F with IAA

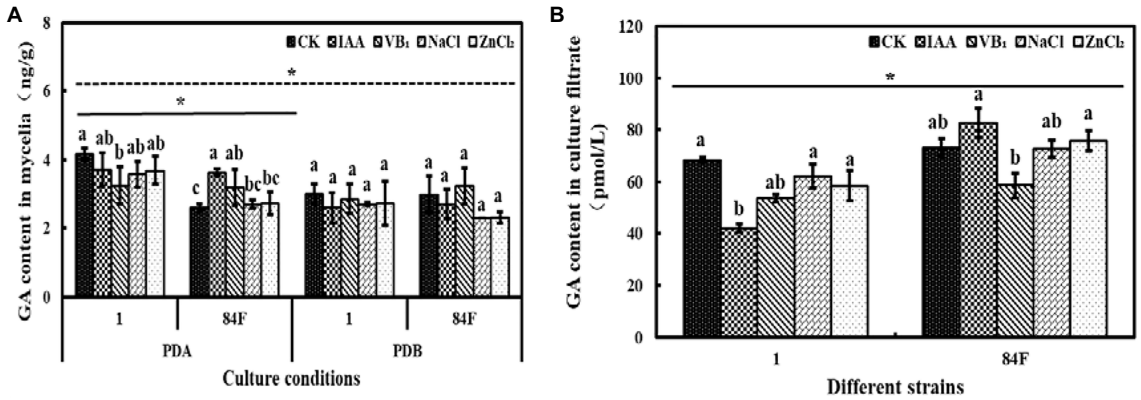

FIGURE 6 | Gibberlic acid content of *E.* sinensis under different treatments for 4 weeks. **(A)** is GA content in mycelia, and **(B)** is GA content in culture filtrate. The same as **Figure 1**.

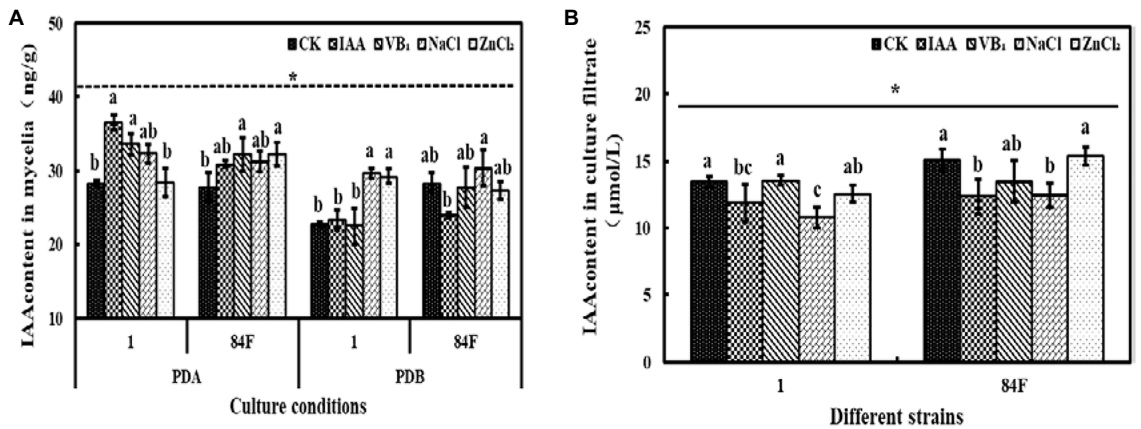

FIGURE 7 | Indole-3-acetic acid content of *E.* sinensis under different treatments for 4 weeks. **(A)** is IAA content in mycelia, and **(B)** is IAA content in culture filtrate. The same as **Figure 1**.

and $ZnCl_2$ treatments were significantly higher ($p < 0.05$) than those with VB_1 treatment (**Figure 6**).

IAA Content

Culture conditions had significant effects ($p < 0.05$) on IAA content in mycelia of the two *E. sinensis* strains. The IAA content in mycelia of *E. sinensis* strains on PDA was significantly higher ($p < 0.05$) than that in PDB (**Figure 7**).

IAA content in mycelia of the two strains on PDA and in PDB was not significantly different ($p < 0.05$). IAA contents in the culture filtrate of strain 84F were significantly higher ($p < 0.05$) than those of strain 1 (**Figure 7**).

Exogenous additives had significant effects ($p < 0.05$) on the IAA content of the two strains. On PDA with IAA and VB_1 treatments, IAA contents of strain 1 were significantly higher ($p < 0.05$) than those with control and $ZnCl_2$ treatments. In PDB, IAA content in mycelia of strain 1 with NaCl and $ZnCl_2$ treatments was significantly higher ($p < 0.05$) than those with control, IAA, and VB_1 treatments. Indole-3-acetic acid content in mycelia of strain 84F with NaCl treatment was significantly higher ($p < 0.05$) than those with IAA treatment. Indole-3-acetic acid content in the culture filtrate of strain 1 with control and VB_1 treatments was significantly higher ($p < 0.05$) than those with IAA and NaCl treatments. Indole-3-acetic acid content in the culture filtrate of strain 84F with control and $ZnCl_2$ treatments was significantly higher ($p < 0.05$) than those with IAA and NaCl treatments (**Figure 7**).

The Percentage of Hormone Content in the Culture Filtrate in Total Content Secreted

The percentages of CTK, ABA, and GA contents in the culture filtrate in total contents secreted by these two strains were significantly different ($p < 0.05$). The percentage of strain 84F was significantly higher ($p < 0.05$) than that of strain 1 (**Table 2**).

Exogenous additives had significant effects ($p < 0.05$) on the percentage of IAA content in the culture filtrate in total content secreted by strain 1. The percentage of strain 1 with control and VB_1 treatments was significantly higher ($p < 0.05$) than

TABLE 2 | Percentage of hormone content in the culture filtrate in total content (%).

Treatment	Cytokinins (CTK)		Abscisic acid (ABA)		Gibberlic acid (GA)		Indole-3-acetic acid (IAA)	
	1	84F	1	84F	1	84F	1	84F
CK	40.17a	50.41a	36.03a	49.26ab	44.23a	46.28b	50.86a	48.24a
IAA	42.31a	44.80b	37.37a	40.17ab	36.23a	51.59a	47.03b	47.38a
VB'	41.16a	43.56b	39.60a	43.89ab	38.80a	39.13a	51.41a	45.95ab
NaCl	37.38a	51.63a	40.23a	53.96a	44.21a	52.00ab	38.91d	41.77b
ZnCl'	35.75a	44.84b	37.78a	38.21b	43.14a	53.15a	42.89c	49.73a
	*		*		*			

**in the table indicates significant differences between different strains ($p < 0.05$).*
Different lowercase letters in the table indicate significant differences between different exogenous additives ($p < 0.05$).

that with the other four treatments; the percentage of strain 1 was significantly different ($p < 0.05$) and changed according to the following order of additives: CK, $VB_1 > IAA > NaCl > ZnCl_2$. Exogenous additives had significant effects ($p < 0.05$) on the percentage of CTK content in the culture filtrate in total content secreted by strain 84F ($p < 0.05$). The percentages of CTK content in the culture filtrate in total content secreted by strain 84F with control and NaCl treatments were significantly higher ($p < 0.05$) than that with IAA, VB_1, and $ZnCl_2$ treatments. The percentage of ABA content in the culture filtrate in total content secreted by strain 84F with NaCl treatment was significantly higher ($p < 0.05$) than that with $ZnCl_2$ treatment and was not significantly different with control, IAA, and VB_1 treatments. The percentage of GA content in the culture filtrate in total content secreted by strain 84F with VB_1 treatment was significantly higher ($p < 0.05$) than that with IAA, NaCl, and $ZnCl_2$ treatments. The percentage of IAA content in the culture filtrate in total content secreted by strain 84F with NaCl treatment was significantly lower ($p < 0.05$) than that with the other treatments (**Table 2**).

DISCUSSION

The endophyte strains isolated from different host ecotypes showed a rich diversity in morphological and growth characteristics (Wei et al., 2010). Ahlholm et al. (2002) showed that host genotypes, along with prevailing environmental conditions, influenced the genetic variation of endophytes. Endophyte strains isolated from a globally distributed collection of perennial ryegrass accessions presented rich gene diversity after evaluation with simple sequence repeat markers (van Zijll De Jong et al., 2008a, 2008b). The genetic polymorphism of grass endophytes is beneficial to select non-toxic strains that do not produce toxic alkaloids to livestock. These selected endophytes were artificially inoculated into grass and established a new grass-endophyte symbiosis that is both stress-resistant and non-toxic to domestic animals and, therefore, improves the quality of grasses and ensures animal safety (Johnson et al., 2013; Young et al., 2013). Yang et al. (2011) found differences in the culture characteristics and growth rates of various endophyte strains isolated from wild *F. sinensis* seeds from Sangke and Ganjia grasslands in the Gansu Province, China (Yang et al., 2011). Significant diversity was observed among the five *E. sinensis* endophyte strains isolated from different *F. sinensis* ecotypes on PDA with a variety of additives at different growth rates (Wang, 2019). The results of the present study support these findings and indicated that *E. sinensis* strains are biodiverse, and the growth and physiological characteristics of *E. sinensis* are affected by host ecotypes. The selection of appropriate markers for genetic polymorphism analysis of *E. sinensis* strains was conducted to reveal the relationship between their growth rate, physiological changes, and host genotype.

The endophyte growth is influenced not only by host genotype, environmental conditions but also by culture conditions (Ahlholm et al., 2010; Wei et al., 2010). We studied the growth of five *E. sinensis* endophyte strains on PDA with different additives and found that PGRs (VB_1, VB_5, VB_9, IAA, GA_3, and KT-30) promoted fungal growth, whereas ions (Na^+, Cd^{2+}, Cr^{6+}, and Zn^{2+}) inhibited it (Wang, 2019). The growth of four *E. sinensis* strains with 30 mg/l VB_1 and five *E. sinensis* strains with 20 mg/L IAA significantly increased ($p < 0.05$), whereas the five strains had the strongest resistance with 0.4 mol/L Na^+ and 750 mg/L Zn^{2+}. Therefore, the four different treatments which included 30 mg/L VB_1, 20 mg/L IAA, 0.4 mol/L NaCl, and 750 mg/L $ZnCl_2$ in this study were set up based on previous experiments and were initiated to determine the response mechanism of these two strains to different additives.

Numerous studies have shown that IAA and VB_1 promote mycelial growth of fungi (Ren et al., 2007; Li et al., 2016; Luo et al., 2021). High concentrations of NaCl and $ZnCl_2$ inhibited the colony diameter of *E. gansuensis* and *Penicillium chrysogenum*, respectively (Jin, 2009; Wang et al., 2020). The present results are similar to those of other studies. Numerous reports have indicated that the application of PGRs, including phytohormones, promotes cell division and enhances plant and microbe growth (Naeem et al., 2004). However, vitamins only play a regulatory role in plant metabolism to maintain normal central metabolic processes (Liao et al., 2018). In this experiment, compared with the control treatment, the promotion effect of IAA treatment on *E. sinensis* was higher than that of VB_1, which may be related to the different effects of vitamins and plant hormones in the organism. The resistance of *E. sinensis* to Na^+ was stronger than that of Zn^{2+}, indicating that *E. sinensis* has different resistance to different metal ions.

Endophytes produce numerous antioxidant compounds that may play roles in enhancing stress tolerance (Schulz et al., 2002; Malinowski and Belesky, 2006; Yuan et al., 2010). Most abiotic (desiccation and/or rehydration, nutrient limitation, and UV radiation) and biotic stresses (pathogens and insect herbivory) induce reactive oxygen species (ROS) production (Beckett et al., 2005). Cells need appropriate systems to allow rapid removal of ROS. Previous studies have found that the optimal zinc concentration in the medium may improve *Lentinus edodes* mycelium growth and the zinc content of the *L. edodes* mycelium, and increase antioxidant enzyme activities (Gang et al., 2017). The antioxidant capacity of endophytes from *Myricaria laxiflora* was extraordinarily high under mild saline-alkali stress (Gao et al., 2016). The present study found that the two *E. sinensis* culture filtrates showed high free radical scavenging ability with NaCl and $ZnCl_2$ treatments, which demonstrated that the antioxidant capacity played an important role during stress resistance of *E. sinensis*. The scavenging ability of superoxide anion radicals and hydroxyl radicals of strain 84F was higher than that of strain 1 under different treatments, which could be used to further investigate the natural antioxidants of the two *E. sinensis* strains.

Potato dextrose broth is currently widely used in the industrial production of medicinal and edible fungi because of the short fungal production cycle (Yang et al., 2000; Huang and Zhang, 2005). In the present study, the mycelial biomass and hyphae diameter in PDB were generally higher than those on PDA. These differences confirmed that PDB medium has the advantage of uniform distribution of nutrients during microbial cultivation, which is conducive to full contact and absorption of nutrients by mycelia cells, and eventually increases the production of mycelia and nutrients. Therefore, PDB can also be applied to enrich more *E. sinensis* mycelium.

Hormones are naturally occurring organic molecules that regulate plant growth and its developmental processes (Costacurta and Vanderleyden, 1995; Hoyerova et al., 2006; Berger et al., 2007) and present in a wide variety of organisms, including fungi (Battista et al., 1990; Zhang et al., 1999; Yue et al., 2000; Yuan et al., 2004; Nambara and Marionpoll, 2005; Hartung, 2010; Spichal, 2012; Ban, 2013). In the present study, IAA, CTK, IAA, and GA_3 in mycelia and fermentation of two *E. sinensis* strains under five treatments were quantified, suggesting that the *E. sinensis* strain produces growth-promoting hormones (Lee, 1990; Abd, 1997; Deotale et al., 1998; Klingler et al., 2011; Chanclud et al., 2016). Previous studies have found *Epichloë* endophyte change hormones to improve host stress tolerance (Battista et al., 1990; Saikkonen et al., 2004; Xia et al., 2018). In this study, hormones were detected in both mycelia and fermentation, indicating that the hormone concentration increased in E+ plants may be also adjusted by endophyte. In most case, the percentages of hormone contents in the culture filtrate in total contents secreted by *E. sinensis* were about 50%. These results provide a basis for the development and utilization of *E. sinensis* strains. In addition, host ecotypes, culture conditions, and exogenous additives affected the hormone content of the strains. However, these effects were not consistent.

Maintaining a constant intracellular K^+ and Na^+ balance is essential for metabolic processes in cells (Zhu, 2003). Restriction of the transport of Na^+ and increase in the K^+ concentration to ensure a high cytosolic K^+/Na^+ ratio are very important for cells to tolerate stress (Berthomieu et al., 2003; Cuin et al., 2003). Ca^{2+} is essential for selective ion transport mechanisms and for the maintenance of K^+ influx and Na^+/K^+ selectivity. This study is one of the few studies on ion response of *E. sinensis* in various media with exogenous additives. Our results showed exogenous additives had effects on Na^+, K^+, and Ca^{2+} contents and the Na^+/K^+ ratio of these two strains and the effects on two strains were inconsistent. This finding suggested *E. sinensis* may adapt to changing environment by regulating ion contents. Compared with other treatments, Na^+ content in mycelia of the two strains was not significantly different or significantly reduced with the NaCl treatment. This may be because organisms selectively absorb or transport ions to reduce their stress damage and maintain normal physiological metabolism. Therefore, exogenously added Na^+ might remain in the culture filtrate. Ion contents on PDA and in the culture filtrate should be further studied to understand the resistance mechanism of *E. sinensis* strains.

CONCLUSION

Our results demonstrate that *E. sinensis* strains isolated from different host ecotypes showed a rich diversity in physiology and biochemistry characteristics in various media with exogenous additives. *E. sinensis* strain might adapt to environmental changes by changing its antioxidant capacity or intracellular ion content. Additionally, PDB culture with IAA and VB_1 additives was the optimal enrichment conditions for *E. sinensis* mycelia and the two *E. sinensis* strains produced IAA, ABA, GA, and CTK to a certain level under different treatments. Collectively, our findings provide a theoretical basis for fully understanding *E. sinensis* and obtain more reference for utilization.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PT designed the experiments. YL did the experiment and analysis. All authors wrote the manuscript, contributed to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.726935/full#supplementary-material

Supplementary Table S1 | Univariate analysis of general linear models was employed to estimate the effects of single factor and their interaction on hypha diameter and mycelia biomass of *Epichloë sinensis* strains in the present study.

Supplementary Table 2 | Univariate analysis of general linear models was employed to estimate the effects of single factor and their interaction on cytokinins, abscisic acid, gibberlic acis, and indole-3-acetic acid contents of *E. sinensis* strains in the present study.

Supplementary Table 3 | Univariate analysis of general linear models was employed to estimate the effects of single factor and their interaction on pH, total antioxidant capacity, scavenging abilities of superoxide anion radical and hydroxyl radical, and ion contents of *E. sinensis* strains in the present study.

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