Effect of sodium selenite concentration and culture time on extracellular and intracellular metabolite profiles of Epichloë sp. isolated from Festuca sinensis in liquid culture

lianyu zhou (zly7604@163.com)
Qinghai Normal University https://orcid.org/0000-0002-3394-4783
Feng Qiao
Qinghai Normal University
Xuelan Ma
Qinghai Normal University School of Geographical Sciences
Jiasheng Ju
Qinghai Normal University
Qiaoyu Luo
Qinghai Normal University

Research Article

Keywords: Epichloë sp. from Festuca sinensis, metabolomics, selenium, culture time

Posted Date: April 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1524555/v1

Abstract

Selenium (Se) is a member of trace elements which are critical for proper functioning of an organism, and is one of the abiotic stressors which affect living organisms growth and metabolite. In this study, *Epichloë* sp. from *Festuca sinensis* was submitted to increasing Na_2SeO_3 concentrations (0, 0.1, and 0.2 mmol/L) in the liquid media for 8 weeks. *Epichloë* sp. mycelia and fermentation broth were collected by centrifuging at 4, 5, 6, 7, 8 weeks of cultivation, respectively. About 157 and 198 metabolites were respectively determined in fermentation broth and mycelia using gas chromatography-mass spectrometer (GC-MS) analysis. Diverse changes in extracellular and intracellular metabolites were observed as *Epichloë* sp. exposured to selenite conditions and cultivation time. Eight common contributed metabolites (alanine, valine, isoleucine, glycine, serine, aminomalonic acid, 4-aminobutyric acid, and phenylalanine) were determined in fermentation broth and mycelia using principal component analysis (PCA). Some metabolites had highly accumulated in fermentation broth while others decreased after different time of exposure to Se as compared to the control media. However some metabolites were presented at lower concentrations in mycelia cultivated at selenite concentrations. These metabolites changed were involved in amino acids, carbohydrates, organic acids, fatty acids, and nucleotides under Se conditions, as well as ones over time. Based on these results, we conclude that selenite concentrations and culture time influenced the extracellular and intracellular metabolite profiles of *Epichloë* sp. from *F. sinensis*.

Introduction

Selenium (Se) is one of the essential metalloid elements in living organisms, and plays a key role in the biosynthesis of the most important selenoenzymes, including glutathione peroxidase, deiodinase iodothyronine, and thioredoxin reductase (Kieliszek and Błażejak, 2016). Microorganisms are capable of converting inorganic selenium into less toxic and more bio-available organic forms (Tie et al., 2017; Zhao et al., 2004; Kieliszek et al., 2015). Differences in the content and availability of selenium causes differences in the growth and metabolic processes of microorganisms (Dong et al., 2012; Čertík et al., 2013; Kieliszek et al., 2019a). Recent studies have indicated that some fungi present metabolic mechanisms to tolerate Se, including lipid and amino acid metabolism (Kieliszek and Dourou, 2021; Kieliszek et al., 2019b). For example, Xu et al. (2020) has reported that 200 mmol/L of Na_2SeO_4 inhibits endophytic bacterial growth, and the endophytic *Herbaspirillum* sp. is capable of transforming through reduction. Based on its function, the application of microbial agents including endophytic microbes would help remediate soils polluted with selenium.

Microorganisms generally encounter a fluctuating condition in substrate during growth, which affect their metabolism and subsequently their physiology. The dynamic response intracellular and extracellular metabolites of *Aspergillus niger* to high or low glucose concentrations has been observed by Wang et al. (2019). Some studies have verified that microbial cells secrete many metabolites to the extracellular medium without intracellular accumulation in response to environmental factors (Han et al., 2013; Granucci et al., 2015). Metabolomic analysis is an important approach commonly used to illustrate real-

time picture of cellular responses, and it also provides the most direct representation of cellular phenotype in response to environmental changes (Pinu et al., 2018).

Endophytes and plants form a symbiosis together (Nazir and Rahman, 2018), and endophytes can promote the growth and selenium accumulation of plants (Lindblom et al., 2018; Xu et al., 2020). Endophytic *Epichloë* species provides grasses with protection against biotic and abiotic stresses (Johnson et al., 2013). To date, many studies have described *Epichloë* species based on morphology, physiological and biochemical properties, host specificity, and phylogenetic analyses (Li et al., 2008; Leuchtmann et al., 2014; Chen et al., 2015; Yi et al., 2018), meanwhile, the ability of *Epichloë* species resistance to stress has been related to host adaptability (Wei et al., 2012).

Festuca sinensis is an important perennial cool-season grass species in cool and semi-arid regions of China. This grass species is frequently infected with an asexual symptomless *Epichloë* sp. which has been shown to enhance the fitness of grass host to stressors such as drought, waterlogging, cold, and pathogens (Peng et al., 2013; Wang et al., 2017; Zhou et al., 2015a, 2015b; 2021), and to produce alkaloids in the host tissues (Tian et al., 2018; Lin et al., 2019). Research on *Epichloë* sp. from *F. sinensis* has been confined to morphology, physiology, phylogeny, and bioactivity in previous studies, and *Epichloë* sp. from *F. sinensis* is identified as *Epichloë sinensis* (Jin et al., 2009; Zhou et al., 2015b; Tian et al., 2020). However, the effect of Se on the biochemical and physiological processes of endophytic microorganisms is scarce.

The current study used a metabolomics approach to deduce the metabolic changes that occurred in *Epichloë* sp. extracellular and intracellular subjected to different selenite concentrations for 8 weeks. Studies on the metabolic mechanisms by which endophytes metabolize selenium are critical in the case of understanding their metabolic overflow and selenium-tolerance mechanism.

Materials And Methods

Epichloë sp. strain

Epichloë endophyte strain, which was maintained on Potato Dextrose Agar media (potato infusion 200 g, dextrose 20 g, agar 20 g, distilled water 1000 mL), was obtained from Key Laboratory of Medicinal Plant and Animal Resources of the Qinghai-Tibetan Plateau, Qinghai Normal University (Zhou et al., 2015b).

Fungal cultivation

Experiments were carried out in 250 mL Erlenmeyer flasks. Three pieces (4 mm diameter) of mycelial agar plugs removed from the edge of an *Epichloë* sp. colony were placed into 250 mL Erlenmeyer flasks with 100 mL PD broth medium (potato infusion 200 g, dextrose 20 g, distilled water 1000 mL), followed by shaking (130 r/min) continuously for 15 days at 25 ± 1℃. Then 5 mL of this culture was added to 250 mL culture flasks containing 95 mL fermentation medium (sucrose 30 g, yeast extract 2.5 g, peptone 1 g, distilled water 1000 mL, natural pH) supplemented with final concentrations of 0, 0.1 or 0.2 mmol/L

Na_2SeO_3. All cultures were incubated at a fixed temperature of 25±1℃. The cultivation was carried out in triplicates for each time point. After that, the cultures were centrifuged at 4000 r/min for 10 min to obtain the mycelia and fermentation broth. The mycelia were washed twice with deionized water, immediately quenched in 200 μL methanol (−20℃), and stored at −80℃ for determination of metabolites.

Metabolite extraction and derivatization

100±1 μL fermentation broth in a 1.5 mL tube was extracted by addition of 350 μL pre-cold methanol and 10 μL internal standard (adonitol, 0.5 mg/mL stock). After samples were vortexed for 30 s, followed by ultrasonication for 10 min in an ice bath, and the extract was centrifuged at 4℃ for 15 min at 10000 r/min. Then, 100 μL supernatant was transferred to a fresh tube, and taken 30 μL from each sample and pooling as QC sample, and dried in a vacuum concentrator without heating. The pellet was resuspended in 60 μL of methoxyamination hydrochloride (20 mg/mL in pyridine) and incubated at 80℃ for 30 min. Subsequently, 80 μL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was added to the sample aliquots, incubated at 70℃ for 1.5 h, and 5 μL fatty acid methyl ester (FAMEs) (in chloroform) was added to the QC sample when cooling to the room temperature. Fermentation broth metabolites were carried out in triplicates.

A total of 10±1 mg of lyophilized mycelial material was transferred into a 2 mL tube, and 450 μL of pre-cold 3:1 (v/v) methanol:chloroform. After the mixture was vortexed for 30 s and homogenized with ball mill for 4 min at 35 Hz, treated by ultrasound for 5 min in an ice bath, and the extract was centrifuged at 4℃ for 15 min at 10000 r/min. Then, the supernatant was very carefully transferred into a fresh tube, and taken 40 μL from each sample and pooling as QC sample, and dried in a vacuum concentrator without heating. The pellet was resuspended in 30 μL methoxyamination hydrochloride (20 mg/mL in pyridine) and incubated for 30 min at 80℃. Subsequently, 40 μL of the BSTFA regent (1% TMCS, v/v) was added to the sample aliquots, incubated for 1.5 h at 70℃, and 5 μL FAMEs (in chloroform) was added to the QC sample when cooling to the room temperature. Mycelial metabolites were analysed in two replicates.

GC-TOF-MS analysis

The analysis was performed on an Agilent 7890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer and a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m×250 μm inner diameter, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). A 1.0 μL aliquot of sample was injected in splitless mode. The initial temperature was kept at 50℃ for 1 min, then raised to 310℃ at a rate of 10℃/min and kept for 8 min at 310℃. Helium was used as the carrier gas at a rate of 1 mL/min. The injection, transfer line, and ion source temperatures were kept at 280℃, 280℃, and 250℃, respectively. Mass spectra collected in the electron impact mode with 70 eV ionization energy were recorded at 12.5 scans/s, with the *m/z* range of 50 to 500 after a solvent delay of 6.25 min.

Data processing and statistical analysis

Chroma TOF 4.3X software of LECO Corporation and LECO-Fiehn Rtx5 database were used for raw peaks exacting, the data baseline filtering and calibration of the baselines, peak alignment, deconvolution analysis, peak identification and integration of the peak area. The identification of metabolites was verified with the help of mass spectrum and retention index. In addition, peaks detected in <50% of QC samples or with an RSD 30% in QC samples were removed.

Relative quantification was estimated using an internal standard for each analyzed metabolite. Multivariate analysis (PCA) was performed with the SPSS software, Version 16.0 (SPSS, Inc., Chicago, IL, USA). To assess the degree of metabolite abundance changes, a heatmap and hierarchical clustering was constructed using MeV software. Using SPSS 16.0 software, data were compared using repeated measurement of one-way analysis of variance and least significant difference (LSD) at significance levels of $P<0.05$ or $P<0.001$.

Results

Identification of metabolites and screening for differential metabolites

Extracellular and intracellular metabolites were respectively measured by GC-MS on the basis of mass spectrum and retention index match, of which the components of amino acid metabolism, glycolysis, the citric acid (TCA) cycle, organic acids, fatty acids, sugars, and sugar alcohols were identified. A of total 380 peaks and about 157 identified metabolites were detected in fermentation broth samples. A of total 440 peaks and about 198 identified metabolites were detected in mycelial samples. The amount of metabolites in mycelia was more than that of fermentation broth, with the common 95 metabolites (Fig. 1). The 62 and 103 metabolites were respectively limited to fermentation broth and mycelia of *Epichloë* sp. .

Principal component analysis of Epichloë **sp. from** F. sinensis

The difference between treated samples from fermentation broth of *Epichloë* sp. at different time points was assessed using principal component analysis (PCA) (Fig. 2). The first principal component produced the greatest contribution (75.79%), and the second principal component accounted for 9.84% of the total. Using this method, a clear distinction was noted between the samples under different culture conditions (cultivation time and Se concentration).

The processing of the metabolomic data with PCA revealed clustering of mycelial samples according to Se concentration and cultivation time (Fig. 3). The first and second principal components produced accounted for 59.25% and 9.03% of the total sample variance, respectively, indicating the obvious difference between the samples under different culture conditions (cultivation time and Se concentration).

We carried out PCA for visualizing the difference among these metabolites of the fermentation broth (Fig. 4). Analysis of 157 metabolites showed that three principal components, the three components representing about 59.78% of the total variance. Metabolites contributing to the first component were lactic acid, alanine, O-methylthreonine, valine, isoleucine, glycine, serine, L-allothreonine, aminomalonic acid, 4-aminobutyric acid, phenyalanine, 5-aminovaleric acid, 4-hydroxybenzoic acid, cis-1,2-dihydronaphthalene, O-phosphorylethanolamine, ornithine, and tagatose. Metabolites contributing to the second component were acetol, glutamine, 1-methylhydantoin, and citraconic aciddegr. Metabolites contributing to the third component were lyxose, diglycerol, and glucose-1-phosphate.

For visualizing the difference among metabolites of mycelia, two primary components were identified using PCA in an analysis of 198 metabolites, with the two components accounting for approximately 39.74% of the total variance (Fig. 5). Metabolites contributing to the first component included pyruvic acid, alanine, sulfuric acid, malonic acid, valine, hydroxyurea, ethanolamine, glycerol, isoleucine, proline, glycine, succinic acid, uracil, fumaric acid, serine, threonine, β-alanine, aminomalonic acid, aspartic acid, oxoproline, 4-aminobutyric acid, glutamic acid, and phenylalanine. Metabolites contributing to the second component included 4-hydroxy-3-methoxybenzyl alcohol, indole-3-acetamide, and dioctyl phthalate.

The eight common contributed metabolites (alanine, valine, isoleucine, glycine, serine, aminomalonic acid, 4-aminobutyric acid, and phenylalanine) were found in both fermentation broth and mycelia (Fig. 4, 5).

Heatmap and hierarchical cluster analysis of Epichloë **sp. from** F. sinensis

In order to identify the different metabolites between fermentation broth samples in response to Se concentrations, all metabolite profiles, consisting of 15 *Epichloë* sp. samples were performed by heatmap and hierarchical cluster analysis (Fig. 6). According to hierarchical clustering analysis of the 157 identified extracellular metabolites, an obvious separation was observed between samples, with 15 samples being clearly grouped into three categories: the first category included two samples (the control samples for weeks 4 and 5 of cultivation), the second category included four samples (both the control and 0.2 mmol/L Na_2SeO_3 treated samples for week 7 of cultivation, as well as the Se-treated samples in the fourth week), and the third category included the remaining nine samples. Additionally, 157 metabolites were divided into two clusters, consisted of 74 and 83 compounds, respectively.

According to hierarchical clustering analysis of the 198 identified intracellular metabolites, an obvious separation was also noted between samples, with 15 samples being clearly grouped into two classes, one category consisted of 5 samples (both the control and treated samples for week 4, as well as control samples between weeks 7 and 8), and the other consisted of the rest 10 samples (Fig. 7) (both the control and treated samples between weeks 5 and 6, as well as treated samples between weeks 7 and 8). Besides, 198 metabolites were divided into two groups, consisted of 61 and 137 compounds, respectively.

Changes in the extracellular and intracellular metabolite profiles of Epichloë **sp. from** F. sinensis **in response to Se**

The changes in identified metabolites of fermentation broth were presented in Tables 1 and 2. Among the 157 identified metabolites, 22 metabolites displayed differential accumulation in the 0.1 mmol/L Se group compared to the CK group (0.1 mmol/L vs. CK), while seventeen metabolites showed decreased levels in the 0.1 mmol/L Se treatment group. We observed an increase in 29 metabolites and a decrease in 13 metabolites in 0.2 mmol/L vs. CK. These findings indicated that Na_2SeO_3 concentrations affected metabolite alterations. Parallel to the cultivation period, 67 metabolites (43%) were found to increase while 70 metabolites (45%) were found to decrease in the fourth week in the Se group compared to the control group (Se vs. CK). In week 5 of cultivation, 87 up-regulated and 58 down-regulated metabolites were detected in Se vs. CK. In week 6, 64 metabolites were up-regulated and 38 ones were down-regulated between Se and control. 77 compounds showed a high accumulation and 43 compounds were suppressed in Se vs. CK during 7 week of cultivation. In the last week of cultivation, 76 compounds showed accumulation at Se concentrations and 54 others decreased. The extracellular samples displayed metabolic differences in terms of cultivation time and Se treatments.

Table 1
Number of increased or decreased metabolite levels among 157 identified metabolites in *Epichloë* sp. fermentation broth from different Se concentration and control groups.

Change	Number of metabolites	
	0.1 mmol/L vs. CK	0.2 mmol/L vs. CK
Increase	22	29
Decrease	17	13

Table 2
Changes in metabolite profiles in *Epichloë* sp. fermentation broth between Se and control groups at weeks 4∼8.

Change in	Number of metabolites				
Se vs. CK	week 4	week 5	week 6	week 7	week 8
Increase	67	87	64	77	76
Decrease	70	58	38	43	54

Changes in metabolites from mycelia of *Epichloë* sp. between Se and control groups were showed in Tables 3 and 4 by comparing. In 0.1 mmol/L vs. CK, three metabolites increased and 35 decreased, whereas two metabolites increased and 29 decreased in 0.2 mmol/L vs 0 mmol/L samples. 43 metabolites were up-regulated and 83 ones were down-regulated in week 4 of cultivation in the Se group compared with control group. In week 5, 21 accumulations and 98 reductions were detected in Se vs. CK. In the 6th week, 22 metabolites were up-regulated and 90 ones were down-regulated between Se and control groups. In 7 week of cultivation, 24 up-regulated and 109 down-regulated metabolites were obtained in Se vs. CK. During the last week of cultivation, 30 metabolites showed a high accumulation

and 91 ones were reduced between Se and control groups. The intracellular samples showed metabolic complexity with regard to cultivation time and Se treatments.

Table 3
Number of increased or decreased metabolite levels among 198 identified metabolites in *Epichloë* sp. mycelia from different Se concentration and control groups.

Change	Number of metabolites	
	0.1 mmol/L vs. CK	0.2 mmol/L vs. CK
Increase	3	2
Decrease	35	29

Table 4
Changes in metabolite profiles in *Epichloë* sp. mycelia between Se and control groups at weeks 4∼8.

Change in Se vs. CK	Number of metabolites				
	week 4	week 5	week 6	week 7	week 8
Increase	42	21	22	24	30
Decrease	86	98	90	109	91

Marked metabolites changed in Epichloë **sp. fermentation broth under selenium condition**

To identify metabolite features that were significantly different between each treatment and the control, One Way ANOVA statistical analysis ($P < 0.001$) were performed. The heatmap visualization showed different trends of metabolite changes for each time of exposure to Se concentrations and different time of exposure to each Se concentration (Figs. 8 and 9). Comparison of the metabolites of fermentation broth exhibited significant differences among culture time and Se concentrations. 64, 40, 36, 49 and 30 distinct metabolites in fermentation broth were identified respectively in weeks 4, 5, 6, 7 and 8 (Fig. 8). Thirteen common metabolites among culture time were pyruvic acid, 2-ketoisovaleric acid, sulfuric acid, O-methylthreonine, glycine, succinic acid, threitol, 3-hexenedioic acid, α-ketoglutaric acid, allose, ribose, ornithine, and tagatose.

At Se values of 0, 0.1, and 0.2 mmol/L, respectively, 67, 47 and 39 marked metabolites in fermentation broth were detected using statistical analysis ($P < 0.001$) during culture process (Fig. 9). There were 25 common metabolites among Se concentrations, such as pyruvic acid, lactic acid, glycolicaccid, 2-ketoisovaleric acid, sulfuric acid, lactamide, ethanolamine, phosphate, glycine, 2-deoxytetronic acid, L-malic acid, threitol, oxoproline, 4-aminobutyric acid, tartaric acid, allose, ribose, xylitol, D-arabitol, putrescine, 3,6-anhydro-D-galactose, glucose-1-phosphate, O-phosphorylethanolamine, lactose, and maltose.

0.1 or 0.2 mmol/L Se addition in the medium significantly increased some amino acids of fermentation broth ($P < 0.001$), such as alanine and citrulline in week 4, tyrosine in week 5, serine in both weeks 4 and 5, oxoproline and phenyalanine in the 4th and 6 to 7 week, isoleucine in the 4th, 7th and 8th weeks, aspartic acid in 4 to 7 week, valine in the 4th, 5th, 7th and 8th weeks, glycine and ornithine over the course of the experiment, compared to those of the control fermentation broth. Besides, there were substantial time changes in several amino acid concentrations of fermentation broth (Fig. 10). Highly significant ($P < 0.001$) effects of culture time were detected for glutamine and O-methylthreonine of the control fermentation broth, proline, tyrosine, and serine of 0.1 mmol/L Se treated fermentation broth, ornithine, phenylalanine, and isoleucine levels of fermentation broth under control and 0.1 or 0.2 mmol/L Se, and glycine, aspartic acid, oxoproline, 4-aminobutyric acid levels of fermentation broth with all experimental media (0–0.2 mmol/L Se). Regarding given treatment time point variance, those levels of both fermentation broth added with 0.1 or 0.2 mmol/L Se were higher in the 4th week than in other time points, however those levels of control fermentation broth were higher in weeks 6 or 8 than in other time points.

During the incubation with all tested selenium concentrations (0.1–0.2 mmol/L Se), the pyruvic acid, 2-ketoisovaleric acid, and α-ketoglutaric acid of the fermentation broth were much more than those in the control medium ($P < 0.001$), whereas succinic acid and 3-hexenedioic acid in fermentation broth were lower than those in the non-treated ones ($P < 0.001$). Furthermore, there was time effect on those compound contents (Fig. 10). In case of all culture medium, significant increases in 2-ketoisovaleric acid and 3-hexenedioic acid were observed after 8 week cultivation. Pyruvic acid for the control group was much higher in the 4th week than after 5 week cultivation, however pyruvic acid in 0.1 mmol/L Se culture broth was greater in the 7th week than in the 8th week and before treatment ($P < 0.001$), and pyruvic acid level of fermentation broth with 0.2 mmol/L Se was sharply increased in the last week of cultivation ($P < 0.001$). A significant increase in succinic acid was observed in the 5th week in experimental media supplemented with 0 and 0.1 mmol/L Se culture media ($P < 0.001$). Control fermentation broth and fermentation broth with 0.1 mmol/L Se for α-ketoglutaric acid levels were significantly ($P < 0.001$) increased in the 4th week compared to other time points, and α-ketoglutaric acid levels in 0.2 mmol/L Se fermentation broth markedly enhanced in the 5th week ($P < 0.001$). The lactic acid concentrations of control fermentation broth were much higher in the 5th week than other time points, lactic acid of 0.1 mmol/L Se treated fermentation broth were greater in weeks 7 and 8 of cultivation than before 6 week cultivation ($P < 0.001$), and lactic acid concentrations for 0.2 mmol/L Se treated fermentation broth peaked in the 7th week. Glycolicaccid of control fermentation broth were significantly increased within 5–6 weeks of cultivation compared to other time points ($P < 0.001$), the glycolicaccid levels in fermentation broth with 0.1 mmol/L Se sharply increased in week 4 ($P < 0.001$), and glycolicaccid levels of 0.2 mmol/L Se treated fermentation broth were much more in 4 and 6 weeks of cultivation than other time points ($P < 0.001$). Highly significant increases in 2-deoxytetronic acid were respectively observed in the 6th week in control experimental media and in the 4th week in experimental media supplemented with 0.1 and 0.2 mmol/L Se culture media ($P < 0.001$). Fermentation broth without addition of Se had significantly higher L-malic acid in week 5 than other culture time ($P < 0.001$), fermentation broth for 0.1 mmol/L Se had much more L-malic acid in 7 to 8 weeks than before treatment ($P < 0.001$), and the

maximum L-malic acid of fermentation broth in the presence of 0.2 mmol/L Se were observed during the last week of cultivation ($P < 0.001$). The tartaric acid levels for the control and 0.2 mmol/l Se-treated fermentation broth were greater in week 8 than before cultivation time ($P < 0.001$), however the tartaric acid levels in 0.1 mmol/L Se-treated fermentation broth were much lower in the 6th week than other time points ($P < 0.001$).

Fermentation broth with Se significantly accumulated tagatose ($P<0.001$) compared to the control fermentation broth, however greatly decreased allose, ribose, and threitol. Many sugars and sugar alcohols of fermentation broth were significantly different across the time points ($P < 0.001$). Fermentation broth for the control and 0.2 mmol/L Se showed higher allose values in the 4th week than after 5 week cultivation, and 0.1 mmol/L Se treated fermentation broth had greater allose in week 5 than other time points ($P < 0.001$). Maltose of fermentation broth without selenium peaked in weeks 4 and 5 of cultivation. The maltose levels of fermentation broth with addition of 0.1 mmol/L Se was significantly higher in the 4th and 7th weeks than other time points, and maltose levels of fermentation broth with addition of 0.2 mmol/L Se was significantly less in the 7th week than before treatment or in the 8th week ($P < 0.001$). Lactose levels of control fermentation broth were significantly higher in week 7 than before treatment and in the last week, fermentation broth in the presence of 0.1 or 0.2 mmol/L Na_2SeO_3 were greater in week 5 than other time points ($P < 0.05$). The ribose concentrations for control, 0.1 mmol/L Se, and 0.2 mmol/L Se treated fermentation broth peaked respectively in the 4th, 5th and 6th weeks ($P < 0.001$). The highest xylitol of the control fermentation broth was observed in the 6th and 8th weeks, the lowest xylitol in fermentation broth with the addition of 0.1 or 0.2 mmol/L selenium were obtained in weeks 6 and 7 of cultivation, respectively. The increase in D-arabitol in all fermentation broth were found to be the highest during 8 week of cultivation.

Marked metabolites changed in Epichloë sp. mycelia under selenium condition

As shown in Figs. 10 and 11, there were significant differences in metabolites of mycelia between culture time and Se concentration. In weeks 4, 5, 6, 7, and 8, respectively, 17, 27, 16, 41 and 6 distinct metabolites were identified in mycelia using LSD ($P < 0.05$). At Se values of 0, 0.1, and 0.2 mmol/L, respectively, 6, 18 and 44 marked metabolites were found in mycelia at a significance level $P < 0.05$. There were not common marked metabolites across the time points ($P < 0.05$) or different Se concentrations. Over the time course, Se promoted N-acetyl-β-D-mannosamine and glucoheptonic acid levels, but inhibited 19 metabolites such as glycolic acid, oxalic acid, 3-hydroxybutyric acid, sulfuric acid, malonic acid, hydroxyurea, dihydroxyacetone, ethanolamine, phosphate, proline, glycine, succinic acid, uracil, 2-deoxytetronic acid, 4-aminobuyric acid, 4-hydroxyphenylethanol, conduritol b epoxide, gentiobiose, and isomaltose.

There were no difference in serine, homoserine, methionine, oxoproline, glutamic acid, phenylalanine, ornithine, N-methyl-alanine, cycloleucine under different Se treatments at a specific culture time. Se at 0.1 and 0.2 mmol/L concentrations significantly increased only citrulline level in the 4th week, but significantly decreased tyrosine level in the 5th and 7th weeks and N-ethylglycine in the 5th week. In the

7th week, alanine, valine, isoleucine, proline, asparagine levels of mycelia with 0.2 mmol/L Se were significantly lower than those of other mycelia, and glycine, serine, threonine and carnitine levels for 0.2 mmol/L Se-treated mycelia were also lower than those of the control mycelia. Similarly, aspartic acid level of mycelia with 0.1 mmol/L Se were lower than those of the control mycelia in week 5. Significant time effects on alanine, valine, isoleucine, proline, glycine, serine, threonine, asparagine, aspartic acid, methionine, oxoproline, glutamic acid, phenylalanine, citrulline levels of mycelia with 0.2 mmol/L Se or ornithine levels of mycelia for 0.1 mmol/L Se were observed ($P < 0.05$, $P < 0.001$). Those amino acid levels of mycelia were increased in week 5 of cultivation compared to other time points.

Se concentration was the significant ($P < 0.05$) effect detected on some organic acids for endophytic mycelia (Fig. 11). In case of both 0.1 and 0.2 mmol/L Se culture medium, increases in 2-ketoisovaleric acid, tartaric acid, and lignoceric acid were observed in the 4th, 7th, and 8th weeks, respectively. Decreases in benzoic acid, galactonic acid, and 3-hydroxybutyric acid were found in mycelia treated with 0.1 and 0.2 mmol/L after 5 week or 7 week cultivation. In the 5th week, α-ketoisocaproic acid, cis-gondoic acid, and palmitic acid levels of mycelia with 0.2 mmol/L Se were significantly greater than those of other mycelia ($P < 0.05$), but arachidonic acid levels of mycelia with 0.1 mmol/L Se were less than those of other mycelia. In week 6, 0.1 mmol/L Se significantly inhibited malonic acid and pipecolinic acid levels in mycelia ($P < 0.05$). Similarly in the 7th week, 3-methyglutaric acid, 2-deoxytetronic acid, and oxalacetic acid levels of mycelia added by 0.2 mmol/L Se were much greater than those of other mycelia ($P < 0.05$). During the last week, 0.2 mmol/L Se enhanced xanthurenic acid levels of mycelia. With respect to the time series, oxalacetic acid levels of the control mycelia were lower in the 4th week than after 5 week cultivation ($P < 0.05$). In addition, mycelia treated with 0.1 mmol/L Se had the lowest 1-monopalmitin in week 4, demonstrated higher lignoceric acid in the 7th week than in the 8th week and before treatment. The lactic acid, tartronic acid, α-ketoisocaproic acid levels of mycelia with addition of 0.2 mmol/L Se were higher in week 4 of cultivation than after treatment ($P < 0.05$). The lowest succinic acid and pipecolinic acid in mycelia with the addition of 0.2 mmol/L selenium were obtained in the last week. The 0.2 mmol/L Se treated mycelia showed lower 2-deoxytetronic acid in weeks 7 and 8 of cultivation than in weeks 4 and 5 of cultivation.

The presence of selenium decreased significantly gentiobiose, leucrose, and turanose in the 4th or 5th weeks. The concentrations of maltose, isomaltose, lactose, and trehalose at dose of 0.1 mmol/L Se were equal to or above the control mycelia at certain time points, and galactose and ribose were significantly lower for 0.1 mmol/L Se-treated mycelia than those of the control mycelia in the 6th and 8th weeks, respectively. When analyzing time shifts in sugars, mycelia treated with 0.1 mmol/L Se had significantly lower sucrose in the 5th week than other time points, and lower maltotriose in the 8th week than before treatment ($P < 0.05$), however higher maltose and trehalose in week 4 than after 5 week cultivation. In addition, the greatest lactose and cellobiose in mycelia added by 0.2 mmol/L Se were obtained in week 4 of cultivation.

There were significant ($P < 0.05$) effects of Se concentration on sugar alcohols in certain time points. Sorbitol of mycelia was higher under 0.2 mmol/L Se than under control and 0.1 mmol/L Se in week 4.

Galactinol and lanosterol derived from mycelia were lower under 0.2 mmol/L Se than under control and 0.1 mmol/L Se in the 7th week. Ergosterol concentration of mycelia with the addition of 0.1 mmol/L Se were significantly higher than those of other mycelia in the 7th week. Additionally, there was significant time effect on some sugar alcohols ($P < 0.05$). Mycelia in the presence of 0.2 mmol/L Se had lower galactinol in weeks 7 and 8 of cultivation than in weeks 4 and 5 of cultivation, lower sorbitol in week 7 than other time points, and higher glycerol in the 5th and 6th weeks than other time points.

Significant effect of Se treatment was detected for some bases and nucleosides for endophytic mycelia. There were marked inosine increases in control mycelia in the 4th week ($P < 0.05$). A small decrease in thymidine of mycelia was observed in the 5 week cultivation in experimental media supplemented with 0.1 mmol/L Se culture media. Selenium at 0.2 mmol/L concentrations culture media significantly reduced thymine and guanosine of mycelia in the 7th or 6th weeks. Moreover, there were substantial time changes in bases and nucleosides. Guanine levels of 0.1 mmol/L Se treated mycelia were less in the 4th week than after 5 week cultivation. Uracil and thymidine levels for 0.2 mmol/L Se treated mycelia peaked in the 5th week. The thymine levels of mycelia treated with 0.2 mmol/L Se was significantly less in the 7th and 8th weeks than in the 4th and 5th weeks ($P < 0.05$). In case of both 0.1 and 0.2 mmol/L Se culture medium, increases in guanosine were observed in the 4 week cultivation.

Discussion

The present study has shown that the changes in extracellular and intracellular metabolite profiles of *Epichloë* sp. at different sodium selenite concentrations during fermentation. Some amino acids, carbohydrates, organic acids, nucleotides, and their metabolites were significantly changed during selenite conditions.

In the experiments described in this paper, the significant ($P < 0.05$, $P < 0.001$) effects of Se concentration were detected for some amino acid levels of mycelia and fermentation broth of *Epichloë* sp.. Some amino acids of mycelia were significantly depressed with increasing Se concentrations (Fig. 10). Those amio acids were increased for the fermentation broth which was in disagreement with the mycelia results (Fig. 8). This may be due to extracellular Se concentration affecting the stability of cell membrane. Selenium at 60 mg/L reduces only the levels of serine and tyrosine in *Cordyceps militaris* mycelia cultivated in a liquid medium, and increased other amino acids (Dong et al., 2012). A recent study demonstrated that some amino acids such as proline, glutamine, alanine, arginine, and metionine, varied between *Candida utilis* and S*accharomyces cerevisiae* enriched with 20 mg Se^{4+}/L culture media, when compared to the control (Kieliszek et al., 2019b). Concentrations of individual amino acids depend on the presence of selenium in the culture conditions, supporting this notion (Suhajda et al., 2000). In addition, our results showed that the alanine, isoleucine, proline, glycine, serine, threonine, asparagine, aspartic acid, oxoproline, methionine, phenylalanine, citrulline, and glutamic acid concentrations of mycelia were significantly affected by time of exposure to 0.2 mmol/L Se ($P < 0.05$), with the maximum levels in the 5th or the 4th weeks (Fig. 11), but mycelial ornithine concentrations at a concentration of 0.1 mmol/L Se were markedly varied over time with maximum in the 7th week. However many extracellular amino acids

showed highly significant dynamic response to low Se conditions. It is likely that selenium contributes to the reductive environment for the catalytic efficiency of many enzymes, and therefore affects the biosynthesis of some amino acids. These findings demonstrate that the amino acid shifts are affected by many factors, such as Se concentration, substrate and time of cultivation, and microorganism species.

Here, most organic acids from fermentation broth that changed were dramatically decreased with increasing Se concentrations, with only a few exceptions. Pyruvic acid is an important intermediate metabolite of the central carbon metabolism that determines the carbon fluxes to the TCA cycle. In this study, the extracellular content of pyruvic acid at selenium condition was highly significant higher than that at the control media (P 0.001). This could be attributed to the TCA cycle, and increased α-ketoglutaric acid level, and underwent further transformations forming ornithine. Thus succinic acid was reduced. However, these organic acids of TCA in mycelia showed no significant change between Se-treated samples. Organic acids are defined as membrane permeable in the undissociated form which are secreted by fungus (Wang et al., 2019). Significant succinic acid concentration for fermentation broth after different times of exposure to Se concentrations from 0 to 0.2 mmol/L and only mycelia after different time of exposure to 0.2 mmol/L Se showed, with the maximum in the 5th week. Similarly, Yang et al. (2016) reported that dynamics of the changes in succinic acid and other TCA organic acids from culture broth of *Aspergillus saccharolyticus* under different pH conditions during the culture period.

The profile of fatty acids are correlated with the duration of culture and selenium concentration (Čertík et al., 2013). The main fatty acid in yeast was oleic acid (C18:1), and *C. utilis* without Se treatment accumulated more oleic acid (C18:1), stearic acid (C18:0), palmitoleic acid (C16:1), as well as myristic acid (C14:0), compared to the selenite-treated culture. However selenium supplementation promoted the abundances of oleic acid, palmitoleic acid, and myristic acid in *S. cerevisiae* (Kieliszek et al., 2019b). Similarly, the addition of selenium to the culture medium caused an increase in the C-18 fatty acid contents of yeast (Čertík et al., 2013). Guan et al. (2010) have found 90 μg/mL Se apparently raised the concentration of arachidonic acid in *Diasporangium jonesianum*, but reduced the levels of myristic acid (14:0), hexadecenoic acids (16:1), and octadecenoic (18:1), and changed slightly the level of other fatty acids, as compared to the control group. Our data collected suggested that selenium at 0.1 and 0.2 mmol/L concentrations significantly increased lignoceric acid (24:0) of mycelia during the last week, and selenium at 0.2 mmol/L concentration significantly increased palmitic acid (16:0) in week 5 and arachidonic acid (20:4) in week 7, respectively. During the fermentation process, few long chain fatty acids were detected in fermentation broth, which implied that long chain fatty acids mainly maintain cell membranes. No significant difference in changes for linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0) and palmitoleic acid (C16:1) were observed in *Epichloë* sp. mycelia, regardless of different Se treated across culture time. This may be due to microorganism species variance affecting fluctuation of fatty acids.

Some carbohydrates are involved in maintaining structural integrity of cells under several adverse environmental conditions. Compared with the control, the carbohydrates from mycelia are more significant in *Penicillium expansum* using the concentration of 20 mg/L Se (Wu et al., 2014). The

addition of 0.1 mmol/L sodium selenite decreases the trehalose content of *S. cerevisiae* (Sharma and Anand, 2006). In this respect, we observed that Se decreased significantly intracellular gentiobiose, leucrose, and turanose levels in the 4th or 5th weeks. The dose of 0.1 mmol/L Se significantly increased trehalose levels in week 4 of cultivation, but respectively decreased galactose and ribose of mycelia in weeks 6 and 8 of cultivation. Moreover, Se highly significantly promote extracellular tagatose and ribose levels. Se may be playing a major role in cell sugar uptake and transport. *Lactobacillus reuteri* CRL1101 could modulate proteins and enzymes involved in sugar synthesis or degradation to adapt to Se conditions (Gómez-Gómez et al., 2019). However, different species have evolved their metabolome for specific metabolites. *Trichoderma harzianum*, *Aureobasidium pullulans*, *Mortierella humilis*, and *Phoma glomerata* treated with 1 mmol/L Na_2SeO_3 had distinct exopolysaccharide levels (Liang et al., 2019).

As previous works have noted, most fungi produce sugars and sugar alcohols to facilitate tolerance of the environmental stress conditions (Ramirez et al., 2004; Neschi et al., 2004). In the case of the fermentations described in the present study, a dose of 0.2 mmol/L Se significantly increased sorbitol in the 4th week, but decreased galactinol and lanosterol levels of mycelia in week 7 when compared to the control samples. Sorbitol as alternative energy source can protect cells against oxidative damage by scavenging off free reactive oxygen radicals (Santivarangkna et al., 2006). Similarly, sorbitol was highly accumulated in the intracellular environment *Streptococcus thermophilus* at stationary phases (Qiao et al., 2020). More interestingly, some studies have shown that lanosterol found in endophytic fungal secondary metabolites has good medicinal potential for treatment of cataract diseases (Zhao et al., 2015; Song et al., 2019). In addition, our results showed that Se highly significantly decreased threitol levels of fermentation broth. In this study, dynamics of the significant changes in glycerol of mycelia under 0.2 mmol/L Se conditions over the culture period were found with the maximum in week 5. However, Ianutsevich et al. (2020) concluded that *Rhizomucor miehei* increased in the level of arabitol and glycerol under osmotic stress, and no significant changes in glycerol between 3 h and 6 h were found. This may be due to time variance affecting the mycelia metabolic capacity. Ergosterol as a vitamin D precursor plays the pivotal role for membrane fluidity. The addition of Se to *Pleurotus ostreatus* and *Ganoderma lucidum* growth media resulted in lower ergosterol contents in fruiting bodies in comparison to the control group (Siwulski et al., 2019). In this respect, ergosterol concentration of mycelia with the addition of 0.1 mmol/L Se were significantly higher than those of other mycelia in the 7th week.

Most purines and pyrimidines in cell take part in the biosynthesis of genetic information carriers (DNA and RNA) or suppliers of energy (ATP and GTP) (Castellanos et al., 2004). In the present study, some intermediates of purine and pyrimidine metabolism of certain Se-treated mycelia including thymine, inosine, 2-dioxyuridine, and guanosine were down-regulated in a certain time point. Whilst a higher thymidine was detected for mycelia exposed to 0.1 mmol/L Se in weeks 5 and 6 of cultivation. The contents of adenosine in *C. militaris* were promoted with increasing Se concentrations (Dong et al., 2012). Results from a study presented by Hu et al. (2019) confirmed high adenosine content was detected in *C. milic* by Se, which regulated purine and pyrimidine metabolism. The results suggest that Se influenced

DNA and RNA synthesis of cells during this whole growing period, thus interfering with mycelial metabolites.

Conclusion

In this study, changes in metabolites in the intracellular and extracellular metabolite profiles of *Epichloë* sp. isolated from *F. sinensis* response to Se over time were determined ($P < 0.05$, $P<0.001$). The extracellular metabolites were considerably less than the intracellular ones. Redox balance seems to drive the active secretion of some intracellutar metabolites. Time series resulted in significant changes of many intracellular metabolites in *Epichlo* sp. mycelia when growing under higher Se concentrations, in contrast, more extracellular metabolites significantly changed occurred in the absence of Se. Moreover, the results presented will help for understanding the relationship between extracellular and intracellular metabolite levels of *Epichloë* sp. which can occur during the course of growth.

Declarations

Author Contributions

Xuelan Ma and Jiasheng Ju performed the experiments. Qiaoyu Luo and Feng Qiao processed the experimental data and performed the analysis. Lianyu Zhou helped in the experimental plan and analyzed data. All authors discussed the results and commented on the manuscript.

Funding Information

This work is financially supported by the National Natural Science Foundation of China (grant numbers 31760697, 2018–2021).

Compliance with ethical standards

Conflict of interest

No conflict of interest was declared.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Castellanos M, Wilson DB, Shuler ML (2004) A modular minimal cell model: purine and pyrimidine transport and metabolism. Proc Natl Acad Sci USA 101(17): 6681–6686 DOI: 10.1073/pnas.0400962101.

2. Čertík M, Breierová E, Oláhová M, Sajbidor J, Marova L (2013) Effect of selenium on lipid alternations in pigment-forming yeasts. Food Sci Biotechnol 22(S): 45–51 DOI: 10.1007/s10068-013-0047-3.
3. Chen L, Li XZ, Li CJ, Swoboda GA, Young CA, Sugawara K, Leuchtmann A, Schardl CL (2015) Two distinct *Epichloë* species symbiotic with *Achnatherum inebrians*, drunken horse grass. Mycologia 107(4): 863–873 DOI: 10.3852/15-019.
4. Dong JZ, Lei C, Ai XR, Wang Y (2012) Selenium enrichment on *Cordyceps militaris* link and analysis on its main active components. Appl Biochem Biotechnol 166(5): 1215–1224 DOI: 10.1007/s12010-011-9506-6.
5. Gómez-Gómez B, Pérez-Corona T, Mozzi F, Pescumab M, Madrid Y (2019) Silac-based quantitative proteomic analysis of *Lactobacillus reuteri* CRL 1101 response to the presence of selenite and selenium nanoparticles. J Proteomics 195: 53–65 DOI: 10.1016/j.jprot.2018.12.025.
6. Granucci N, Pinu FR, Han TL, Villas-Boas SG (2015) Can we predict the intracellular metabolic state of a cell based in extracellular metabolite data? Mol BioSyst 11(12): 3297–3304 DOI: 0.1039/C5MB00292C.
7. Guan XY, Dai CC, Xu YF (2010) Enhancement of polyunsaturated fatty acid production by selenium treatment in polyunsaturated fatty acid-producing fungus. J Am Oil Chem Soc 87(11): 1309–1317 DOI: 10.1007/s11746-010-1610-1.
8. Han T L, Tumanov S, Cannon RD, Villas-Boas SG (2013) Metabolic response of *Candida albicans* to phenylethyl alcohol under hyphae-inducing conditions. PLoS One 8(8): e71364 DOI: 10.1371/journal.pone.00713 64.
9. Hu T, Liang Y, Zhao GS, Wu WL, Li HF, Guo YB (2019) Selenium biofortification and antioxidant activity in *Cordyceps militaris* supplied with aelenate, aelenite, or aelenomethionine. Biol Trace Elem Res 187: 553–561 DOI: 0000-0003-0134-28491,2.
10. Ianutsevich EA, Danilova OA, Kurilov DV, Zavarzin LV, Tereshina VM (2020) Osmolytes and membrane lipids in adaptive response of thermophilic fungus *Rhizomucor miehei* to cold, osmotic and oxidative shocks. Extremophiles 24: 391–401 DOI: 10.1007/s00792-020-01163-3.
11. Jin WJ, Li CJ, Nan ZB (2009) Biological and physiological characteristics of *Neotyphodium* endophyte symbiotic with *Festuca sinensis*. Mycosystema 28(3): 363–369 DOI: 10.13346/j.mycosystema.2009.03.008 (In Chinese, with English abstract).
12. Johnson LJ, de Bonth ACM, Briggs LR, Caradus J, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ, Tapper BA, Simpson WR, Voisey CR, Card SD (2013) The exploitation of *epichloae* endophytes for agricultural benefit. Fungal Divers 60(1): 171–188 DOI: 10.1007/s13225-013-0239-4.
13. Kieliszek M, Błażejak S (2016) Current knowledge on the importance of selenium in food for living organisms: a review. Molecules 21: 609. DOI: 10.3390/molecules21050609.
14. Kieliszek M, Błażejak S, Bzducha-Wróbel A (2019a) Effect of selenium on growth and antioxidative system of yeast cells. Mol Biol Rep 46: 1797–1808 DOI: 10.1007/s11033-019-04630-z.

15. Kieliszek M, Błażejak S, Bzducha-Wróbel A, Kot AM (2019b) Effect of selenium on lipid and amino acid metabolism in yeast cells. Biol Trace Elem Res 187: 316–327 DOI: 10.1007/s12011-018-1342-x.
16. Kieliszek M, Błażejak S, Gientka I, Bzducha-Wróbel A (2015) Accumulation and metabolism of selenium by yeast cells. Appl Microbiol Biotechnol 99(13): 5373–5382 DOI: 10.1007/s00253-015-6650-x.
17. Kieliszek M, Dourou M (2021) Effect of selenium on the growth and lipid accumulation of *Yarrowia lipolytica* yeast. Biol Trace Elem Res 199: 1611–1622 DOI: 10.1007/s12011-020-02266-w.
18. Leuchtmann A, Bacon CW, Schardl CL, White JF, Tadych M (2014) Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. Mycologia 106(2): 202–215 DOI: 10.3852/106.2.202.
19. Li CJ, Nan ZB, Li F (2008) Biological and physiological characteristics of *Neotyphodium gansuense* symbiotic with *Achnatherum inebrians*. Microbiol Res 163(4): 431–440 DOI: 10.1016/j.micres.2006.07.007.
20. Liang XJ, Marie-Jeanne Perez MA, Nwoko KC, Egbers P, Feldmann J, Csetenyi L, Gadd GM (2019) Fungal formation of selenium and tellurium nanoparticles. Appl Microbiol Biot 103: 7241–7259 DOI: 10.1007/s00253-019-09995-6.
21. Lin WH, Kuang Y, Wang JJ, Duan DD, Xu WB, Tian P, Nzabanita C, Wang MN, Li MM, Ma BH (2019) Effects of seasonal variation on the alkaloids of different ecotypes of *Epichloë* endophyte– *Festuca sinensis* associations. Front microbiol 10: 1695 DOI: 10.3389/fmicb.2019.01695.
22. Lindblom SD, Wangeline AL, Valdez Barillas JR, Devibiss B, Fakra SC, Pilon-Smits EAH (2018) Fungal endophyte *Alternaria tenuissima* can affect growth and selenium accumulation in its hyperaccumulator host *Astragalus bisulcatus*. Front Plant Sci 9: 1213 DOI: 10.3389/fpls.2018.01213.
23. Nazir A, Rahman HA (2018) Secrets of plants: endophytes. Int J Plant Biol 9(1): 1–17 DOI: 10.4081/pb.2018.7810.
24. Neschi A, Etcheverry M, Magan N (2004) Osmotic and matric potential effects on growth and sugar alcohol and solute accumulation in *Aspergillus* section *Flavi* strains from Argentina. J Appl Microbiol 96: 965–972 DOI: 10.1111/j.1365-2672.2004.02246.x.
25. Peng QQ, Li CJ, Song ML, Nan ZB (2013) Effects of seed hydropriming on growth of *Festuca sinensis* infected with *Neotyphodium* endophyte. Fungal Ecol 6: 83–91 DOI: 10.1016/j.funeco.2012.08.001.
26. Pinu FR, Granucci N, Daniell J, Han TL, Carneiro S, Rocha I, Nielsen J, Villas-Boas SG (2018) Metabolite secretion in microorganisms: the theory of metabolic overflow put to the test. Metabolomics 14(4): 43– DOI:10.1007/s11306-018-1339-7.
27. Qiao YL, Liu GF, Lv XP, Fan XJ, Zhang YJ, Meng L, Ai MZ, Feng Z (2020) Metabolic pathway profiling in intracellular and extracellular environments of *Streptococcus thermophilus* during pH-controlled batch fermentations. Front Microbiol 10: 3144– DOI: 10.3389/fmicb.2019.03144.
28. Ramirez ML, Chulze SN, Magan N (2004) Impact of osmotic and matric water stress on germination, growth, mycelial water potentials and endogenous accumulation of sugars and sugar alcohols by

Fusarium graminearum. Mycologia 96(3): 470–478 DOI: 10.2307/3762167.

29. Santivarangkna C, Kulozik U, Foerst P (2006) Effect of carbohydrates on the survival of *Lactobacillus helveticus* during vacuum drying. Lett Appl Microbiol 42(3): 271–276 DOI: 10.1111/j.1472-765X.2005.01835.x.
30. Sharma SC, Anand MS (2006) Role of selenium supplementation and heat stress on trehalose and glutathione content in *Saccharomyces cerevisiae*. Appl Biochem Biotechnol 133(1): 1–8 DOI: 10.1385/abab:133:1:1.
31. Siwulski M, Budzyńska S, Rzymski P, Gąsecka M, Niedzielski P, Kalač P (2019) The effects of germanium and selenium on growth, metalloid accumulation and ergosterol content in mushrooms: experimental study in *Pleurotus ostreatus* and *Ganoderma lucidum.* Eur Food Res Tech 245: 1799–1810 DOI: 10.1007/s00217-019-03299-9.
32. Song R, Wang J, Sun L. Zhang YJ, Ren ZH, Zhao BY, Lu H (2019) The study of metabolites from fermentation culture of *Alternaria oxytropis*. BMC Microbiol 19(1): 35– DOI: 10.1186/s12866-019-1408-8.
33. Suhajda A, Hegoczki J, janzso B, Pais I, Vereczkey G (2000) Preparation of selenium yeasts I. Preparation of selenium-enriched *Saccharomyces cerevisiae*. J Trace Elem Med Biol 14: 43–47 DOI: 10.1016/s0946-672x(00)80022-x.
34. Tian P, Kuang Y, Lin WH, Wang JJ, Nan ZB (2018) Shoot morphology and alkaloid content of *Epichloë* endophyte-*Festuca sinensis* associations. Crop Pasture Sci 69: 430–438 DOI: 10.1071/CP17231.
35. Tian P, Xu WB, Li CJ, Song H, Wang MN, Schardl CL, Nan ZB (2020) Phylogenetic relationship and taxonomy of a hybrid *Epichloë* species symbiotic with *Festuca sinensis*. Mycol Prog 19(10): 1069–1081 DOI:10.1007/s11557-020-01618-z.
36. Tie M, Li B, Sun T, Guan W, Liang Y, Li H (2017) HPLC-ICP-MS speciation of selenium in Se-cultivated *Flammulina velutipes*. Arab J Chem 13(1): 416–422 DOI: 10.1016/j.arabjc. 2017.05.012.
37. Wang JJ, Zhou YP, Lin WH, Li MM, Wang MN, Wang ZG, Kuang Y, Tian P (2017) Effect of an *Epichloë* endophyte on adaptability to water stress in *Festuca sinensis*. Fungal Ecol 30: 39–47 DOI: 10.1016/j.funeco.2017.08.005.
38. Wang S, Liu P, Shu W, Li C, Li H, Liu SS, Xia JY, Noorman H (2019) Dynamic response of *Aspergillus niger* to single pulses of glucose with high and low concentrations. Bioresour Bioprocess 6(1): 16– DOI: 10.1186/s40643-019-0251-y.
39. Wei MY, Yin LJ, Jia T, Zhu MJ, Ren AZ, Gao YB (2012) Responses of three endophyte fungi species isolated from natural grass to abiotic stresses. Bot Res 1: 1–7 DOI: 10.12677/br.2012.11001 (In Chinese, with English abstract).
40. Wu ZL, Yin, XB, Lin, ZQ, Bañuelos GS, Yuan LX, Liu Y, Li M (2014) Inhibitory effect of selenium against *Penicillium expansum* and its possible mechanisms of action. Curr Microbiol 69(2): 192–201 DOI: 10.1007/s00284-014-0573-0.

41. Xu X, Cheng W, Liu X, You H, Wu GT, Ding KM, Tu XL, Yang LF, Wang YP, Li YD, Gu HS, Wang XG (2020) Selenate reduction and selenium enrichment of tea by the endophytic *Herbaspirillum* sp. strain WT00C. Curr Microbiol 77: 588–601 DOI:10.1007/s00284-019-01682-z.
42. Yang L, Lübeck M, Ahring BK, Lübeck PS (2016) Enhanced succinic acid production in *Aspergillus saccharolyticus* by heterologous expression of fumarate reductase from *Trypanosoma brucei*. Appl Microbiol Biotechnol 100(4): 1799–1809 DOI: 10.1007/s00253-015-7086-z.
43. Yi M, Hendricks WQ, Kaste J, Charlton ND, Nagabhyru P, Panaccione DG, Young CA (2018) Molecular identification and characterization of endophytes from uncultivated barley. Mycologia 110: 453–472 DOI: 10.1080/00275514.2018.1464818.
44. Zhao L, Chen XJ, Zhu J, Xi YB, Yang X, Hu LD, Ouyang H, Patel SH, Jin X, Lin D, Wu F, Flagg K, Cai H, Li G, Cao G, Lin Y, Chen D, Wen C, Chung C, Wang Y, Qiu A, Yeh E, Wang W, Hu X, Grob S, Abagyan R, Su Z, Tjondro HC, Zhao XJ, Luo H, Hou R, Jefferson J, Perry P, Gao W, Kozak I, Granet D, Li Y, Sun X, Wang J, Zhang L, Liu Y, Yan YB, Zhang K (2015) Lanosterol reverses protein aggregation in cataracts. Nature 523(7562): 607–611 DOI: 10.1038/nature14650.
45. Zhao L, Zhao, GH, Zhao Z.D, Chen P, Tong JY, Hu XS (2004) Selenium distribution in a Se-enriched mushroom species of the genus *Ganoderma*. J Agr Food Chem 52(12): 3954–3959 DOI: 10.1021/jf049965i.
46. Zhou LY, Li CJ, White JF, Johnson RD (2021) Synergism between calcium nitrate applications and fungal endophytes to increase sugar concentration in *Festuca sinensis* under cold stress. Peer J 9: e10568 DOI: 10.7717/peerj.10568.
47. Zhou LY, Li CJ, Zhang XX, Johnson R, Bao GS, Yao X, Chai Q (2015a) Effects of cold shocked *Epichloë* infected *Festuca sinensis* on ergot alkaloid accumulation. Fungal Ecol 14: 99–104 DOI: 10.1016/j.funeco.2014.12.006.
48. Zhou LY, Zhang XX, Li CJ, Christensen MJ, Nan ZB (2015b) Antifungal activity and phytochemical investigation of the asexual endophyte of *Epichloë* sp. from *Festuca sinensis*. Sci China Life Sci 8: 821–826 DOI: 10.1007/s11427-015-4845-0.

Figures

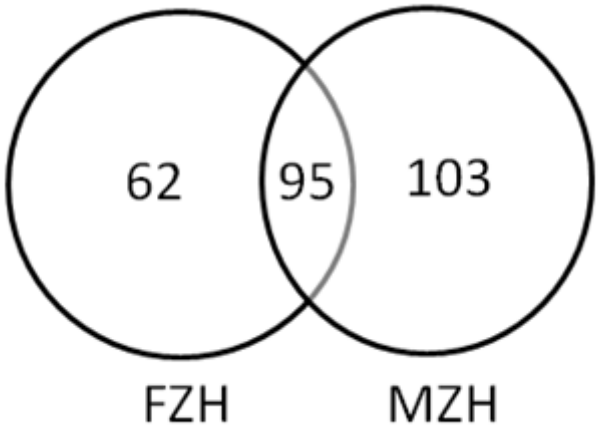

Figure 1

Venn diagram of differentially expressed metabolites from in mycelia (MZH) and fermentation broth (FZH) of *Epichloë* sp. from *F. sinensis* in liquid culture.

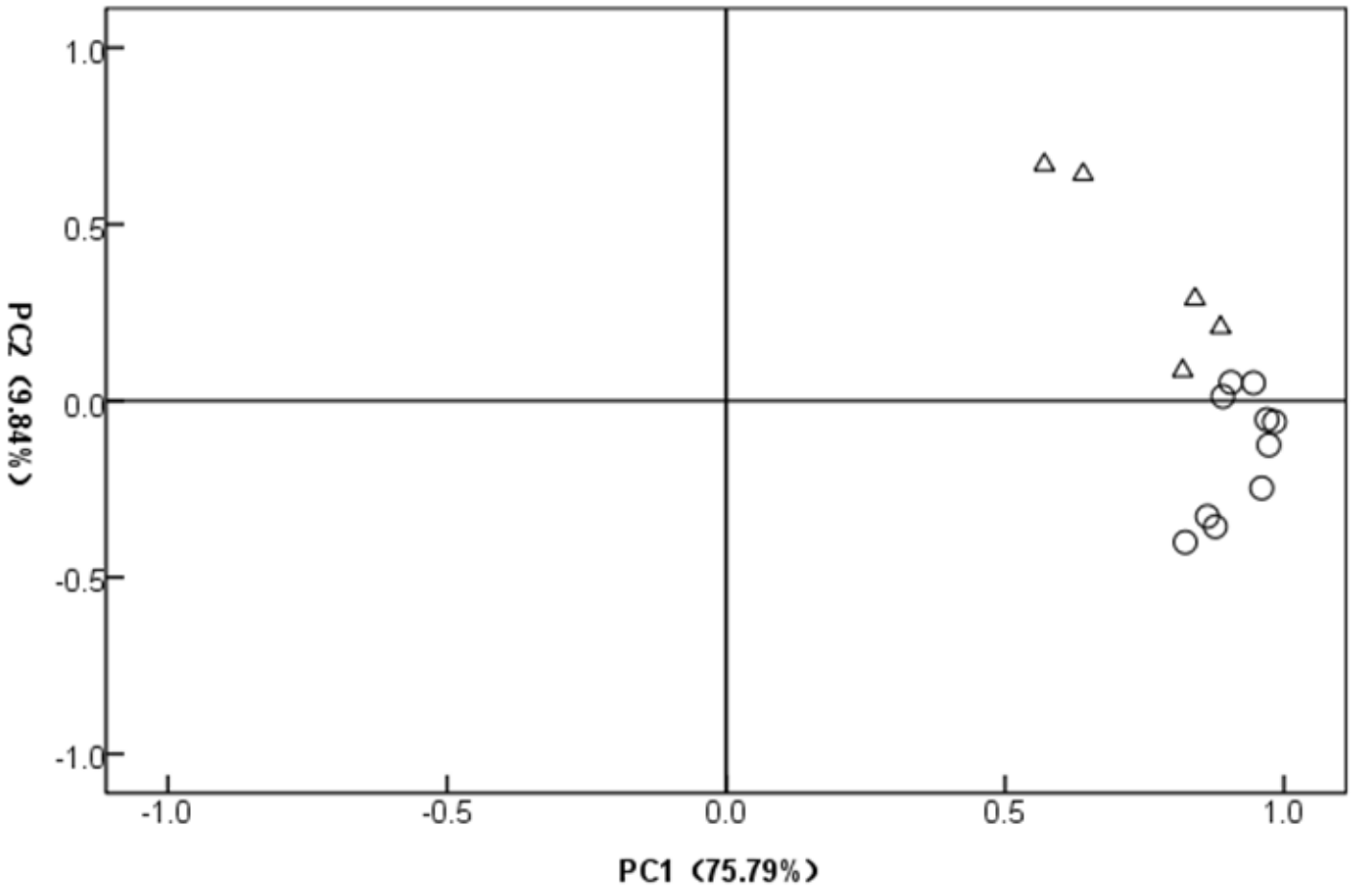

Figure 2

Principal component analysis (PCA) score plot of first and second PCs from 15 fermentation broth samples. PC1: the first principal component; PC2: the second principal component. △ and ● represented control and Se-treated samples, respectively.

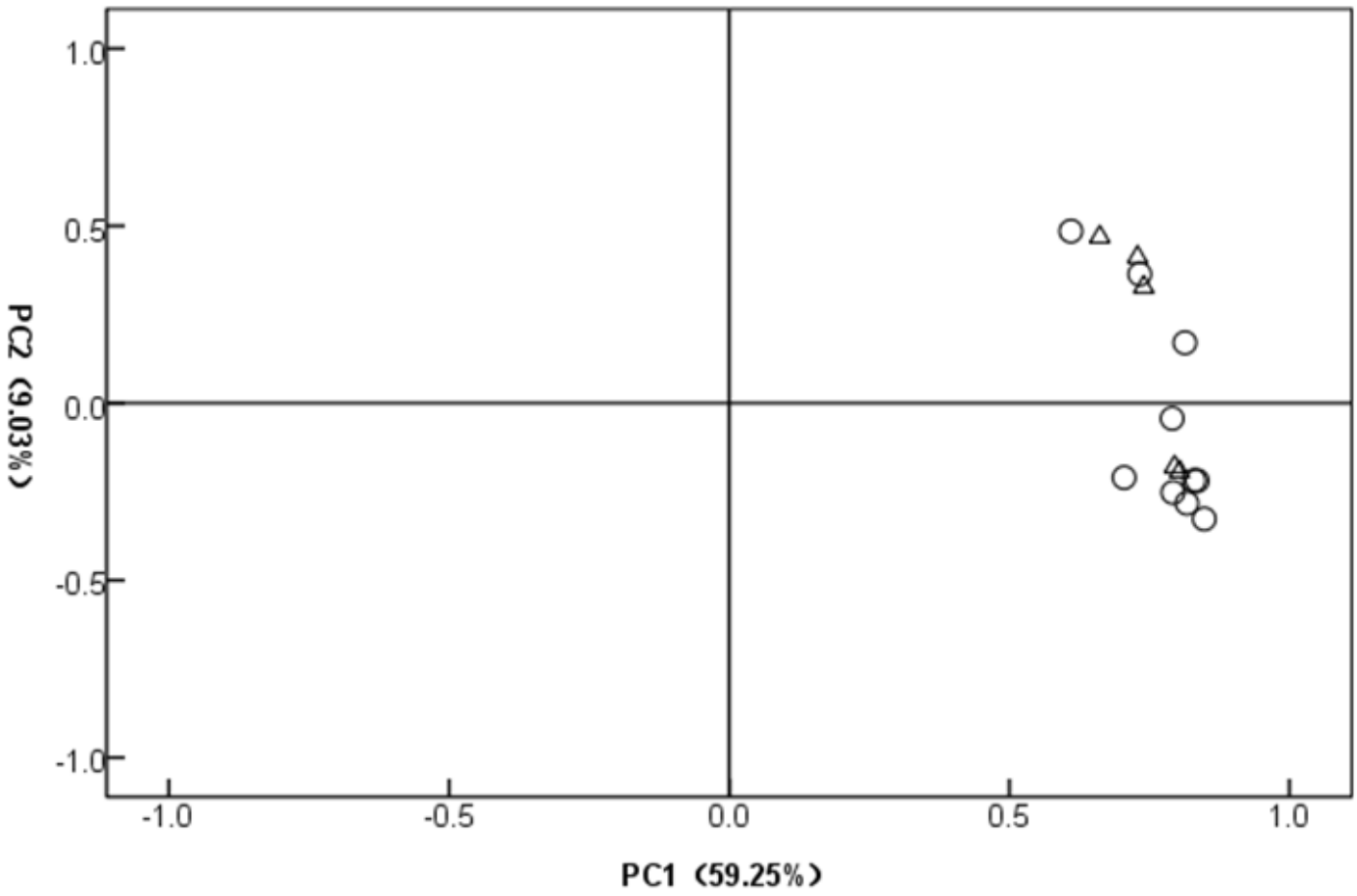

Figure 3

Principal component analysis (PCA) score plot of first and second PCs from 15 mycelial samples. PC1: the first principal component; PC2: the second principal component. △ and ● represented control and Se-treated samples, respectively.

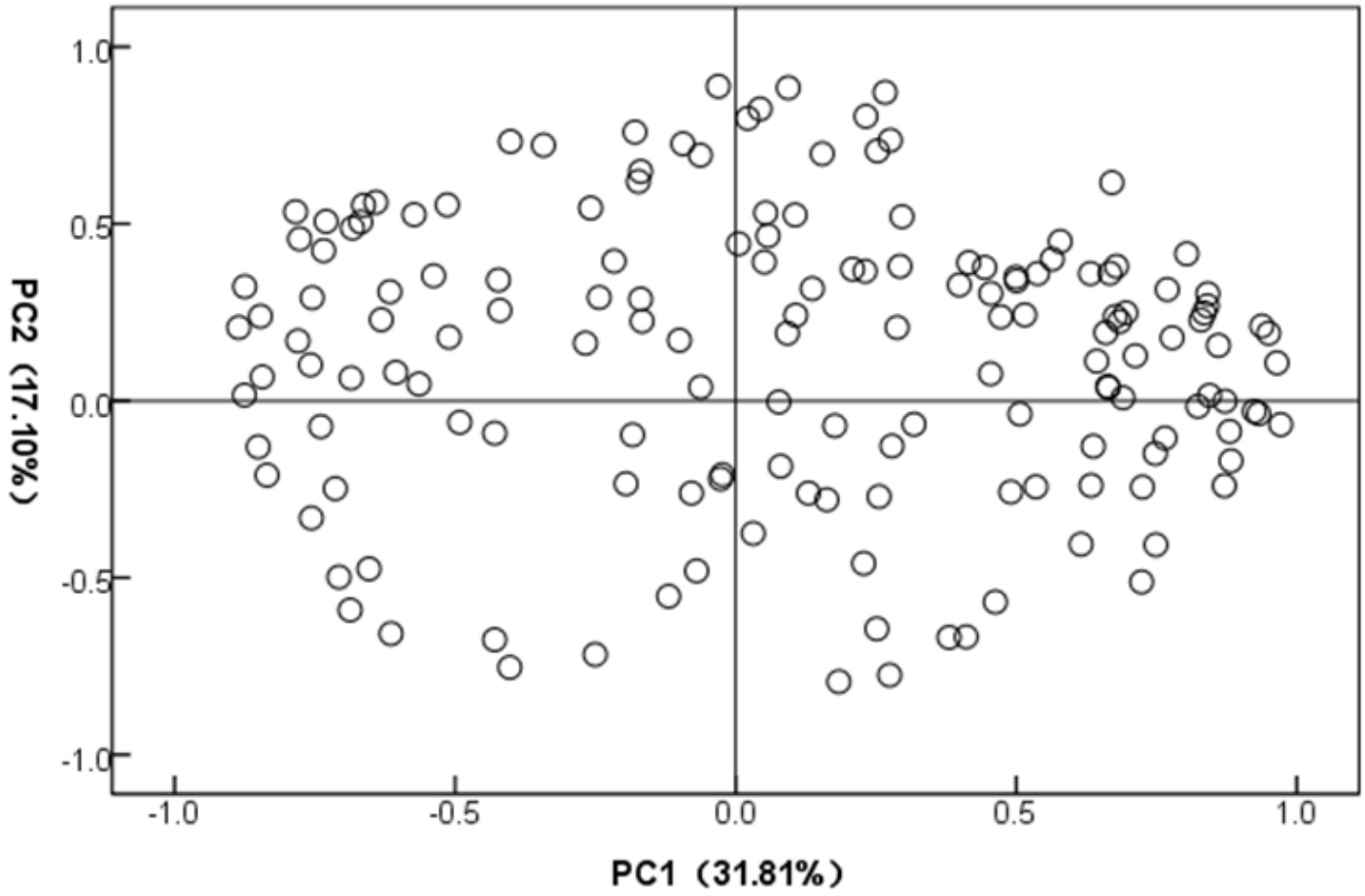

Figure 4

Principal component analysis score plot of first and second PCs from 157 metabolites in the fermentation broth of *Epichloë* sp.. PC1: the first principal component; PC2: the second principal component.

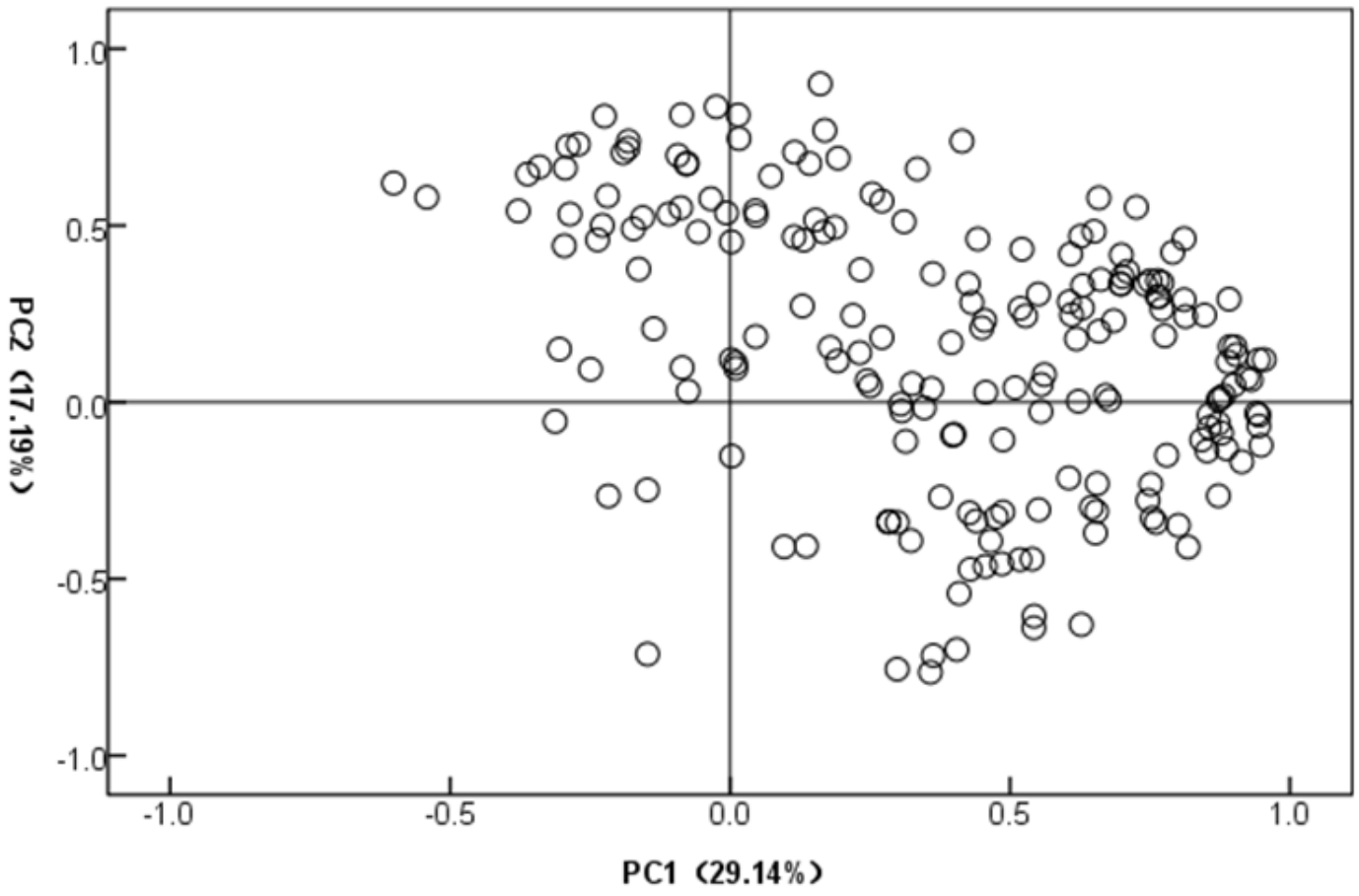

Figure 5

Principal component analysis score plot of first and second PCs from 198 metabolites in the mycelia of *Epichloë* sp.. PC1: the first principal component; PC2: the second principal component.

Figure 6

Heatmap and hierarchical cluster analysis for the 157 metabolites in *Epichloë* sp. fermentation broth. FZH40, FZH41 and FZH42 represented the treated 4 week fermentation broth samples in the presence of 0, 0.1 or 0.2 mmol/L Na_2SeO_3, respectively. FZH50, FZH51 and FZH52 represented the treated 5 week fermentation broth samples in the presence of 0, 0.1 or 0.2 mmol/L Na_2SeO_3, respectively. FZH60, FZH61 and FZH62 represented the treated 6 week fermentation broth samples in the presence of 0, 0.1 or 0.2

mmol/L Na_2SeO_3, respectively. FZH70, FZH71 and FZH72 represented the treated 7 week fermentation broth samples in the presence of 0, 0.1 or 0.2 mmol/L Na_2SeO_3, respectively. FZH80, FZH81 and FZH82 represented the treated 8 week fermentation broth samples in the presence of 0, 0.1 or 0.2 mmol/L Na_2SeO_3, respectively.

Figure 7

Heatmap and hierarchical cluster analysis for the 198 metabolites in *Epichloë* sp. mycelia. MZH40, MZH41 and MZH42 represented mycelia grown for 4 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. MZH50, MZH51 and MZH52 represented mycelia grown for 5 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. MZH60, MZH61 and MZH62 represented mycelia grown for 6 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. MZH70, MZH71 and MZH72 represented mycelia grown for 7 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. MZH80, MZH81 and MZH82 represented mycelia grown for 8 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively.

Figure 8

stamp of the abundance of marked metabolites in *Epichloë* sp. fermentation broth under selenium concentrations among a given culture time. Red and green blocks indicated a higher and lower metabolite levels (see scale bar). Z40, Z41, and Z42 represented fermentation broth for 4 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z50, Z51, and M52 represented fermentation broth for 5 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z60, Z61, and Z62 represented

fermentation broth for 6 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z70, Z71, and Z72 represented fermentation broth for 7 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z80, Z81, and Z82 represented fermentation broth for 8 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively.

Figure 9

Time stamp of the abundance of marked metabolites in *Epichloë* sp. fermentation broth under each selenium concentration. Red and green blocks indicated a higher and lower metabolite levels (see scale bar). Z40, Z50, Z60, Z70, and Z80 represented fermentation broth in absense of Se for 4, 5, 6, 7, and 8 weeks, respectively. Z41, Z51, Z61, Z71, and Z81 represented fermentation broth in the presence of 0.1 mmol/L Se between 4–8 weeks. Z42, Z52, Z62, Z72, and Z82 represented fermentation broth in the presence of 0.2 mmol/L Se during weeks 4–8.

Figure 10

stamp of the abundance of marked metabolites in *Epichloë* sp. mycelia under selenium concentrations among a given culture time. Red and green blocks indicated a higher and lower metabolite levels (see scale bar). Z40, Z41, and Z42 represented mycelia grown for 4 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z50, Z51, and M52 represented mycelia grown for 5 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z60, Z61, and Z62 represented mycelia grown for 6 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z70, Z71, and Z72 represented mycelia grown for 7 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively. Z80, Z81, and Z82 represented mycelia grown for 8 weeks in the presence of 0, 0.1, or 0.2 mmol/L Na_2SeO_3, respectively.

Figure 11

Time stamp of the abundance of marked metabolites in *Epichloë* sp. mycelia under each selenium concentration. Red and green blocks indicated a higher and lower metabolite levels (see scale bar). Z40, Z50, Z60, Z70, and Z80 represented mycelia grown in absense of Se for 4, 5, 6, 7, and 8 weeks, respectively. Z41, Z51, Z61, Z71, and Z81 represented mycelia grown in the presence of 0.1 mmol/L Se between 4–8 weeks. Z42, Z52, Z62, Z72, and Z82 represented mycelia grown in the presence of 0.2 mmol/L Se during weeks 4–8.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterial.docx