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Tree Species and Environmental Factors Determine Fatty Acids Composition of Ectomycorrhizal Mycelia

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By observing the effect of pH and temperature on mycelia growth of eight ECM fungal species from larch (*Larixgmelini* and *L. kaempferi*), we found that only *Boleteuspictus* and *Suillus grevillei*-2 with maximum optimal growth of pH 4.0 and of 25 ℃ belong to the species of narrow range of pH and temperature, while other species varied from pH 4.0 to pH 8.0, and at 10~30℃. Furthermore,all larch species contained four main lipid biomarkers of 18:2ω6,9c, 18:1ω9c, 16:0and 10Me 18:0 TBSA, accounting for up to 80% of relative abundance. The values of 18:1ω9c / 16:0, 16:0/10Me 16:0 and 18:2ω6,9c/TBSA 10Me 18:0 were 1: 1 in all the ECM mycelia, and 5: 1 in limit to ECM mycelia of larch in terms of fatty acids stoichiometry. By multivariate analysis, the fatty acids profiles had clearly separated either between species in larch and in Norway spruce, or between *S. grevillei*-1 in *L. gmelini*and *S. grevillei*-2 in *L. kaempferi*.Taken together, these findings suggested that FA profiles of pure cultured mycelia might have a chemotaxonomic potential in ECM fungi, and that FA composition as well as FA stoichiometry were affected by the host tree species and environmental factors.

Key words: Fatty acids composition, Fatty acids stoichiometry, Larch, Ectomycorrhizalmycelium, Chemotaxonomy.

Belowground Carbon (C) allocation in forest ecosystems represents 30-60% of GPP on a global scale, which provides a pathway of photosynthetic C entering soil via fine roots and mycorrhizal hyphae[1].Contribution of the organic C from fine roots and mycorrhizalmycelia is in the same order of magnitude[2]. However, it is a challenge to unambiguously determine the organic C in separation fine roots from mycorrhiza, which limits our better understanding of belowground carbon flux and prediction for influence of climate change on C balance of forest ecosystems.

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Ectomycorrhizal (ECM) fungi are ubiquitous in temperate and boreal forests, where they represent key components of biogeochemistry cycling processes, in particular, mineral nutrient cycling[3]. The ECM fungi not only uptake nutrients by mineral weathering and soil organic matter decomposition[4], but also enhance fine root lifespan and provide resistance to stresses, such as drought and soil pathogens[5,6]. ECM fungi perform these ecological functions by their mycorrhizalmycelia systems-extramatrical mycelium (EMM) that grows from ECM root tips into the surrounding soil to forage for mineral nutrients and water[7]. Furthermore, EMM from ECM fungi, arbuscularmycorrhizal fungi, saprophytic fungi and fine roots form common mycorrhizal networks (CMNs), which play key roles in

facilitating seedling establishment, improving soil structure and transferring signal between species[8-11]. Recent studies have suggested that EMM or CMNs is a significant pathway for C movement into soil and for C input to belowground food webs, and thus may constitute a considerable belowground sink for carbon sequestration[12-14].

In order to elucidate EMM ecological relevance and describe the C cycling in forest ecosystems, it needs precise measurements of production, standing biomass and turnover of EMM[15]. Among methods, the signature lipid biomarkers of phospholipid fatty acids (PLFAs) are employed in quantification of EMM biomass. Actually, PLFAs have been employed as taxonomic markers for microbial classification[16,17], biomass estimations and community structure analysis[18,19]. Moreover, studying the impact of allelochemicals, herbicides and anaerobically digested sludge on soil microbial community structure has been conducted on basis of PLFA profiles[20-22]. Although the fatty acid-based techniques are used to estimating the EMM biomass of ECM fungi[18], AM fungi in the field[23], few reports on the concentration of the fungal-specific lipid fatty acid in pure cultured mycelia of ectomycorrhizal fungi species, which calls for further studies.

Larch (Pinaceae), containing two species *Larixgmelini* and *L. kaempferi* in North China, is one of the main conifer tree species characterized by a wide natural range, covering north part of China between latitudes 30º and 53ºN under a wide variety of site conditions[24]. The broad latitudinal range of larch from temperate to boreal forests allows for an investigation of EMM traits variation along gradients of temperature, precipitation as well as nitrogen deposition, which should be a valuable model system to assess the mycelia of ectomycorrhizal fungi species on the basis of phospholipid fatty acids. Accordingly, the objectives of this study were (1) to examine ECM fungal species from larch in a wide variety of site conditions, (2) to identify which fatty acids act as the phospholipid biomarkers to classify ECM fungal species in larch, (3) to evaluate the potential use of signature fatty acids for qualitative comparisons at the level of species and community by examining the fatty acids composition in pure cultured mycelia of ECM fungi representing various host trees and various forest locations.

MATERIALS AND METHODS

Chemicals and biological materials

Solvents used in this study were HPLC grade, while other chemicals were analytical grade. Eight ECM fungal species in larch including *Boleteus*sp. (strain EL 8802),*Boletinuspictus*(strain EL 88125), *Cortinarius* sp. (strain EL 8817), *Leccinumscabrum* (strain EL 88134), *Lactariusisulsus* (strain EL 8810), *Suillusaeruginascens*(strain EL 88131), *Suillus grevillei*-1(strain EL 8803), *Suillus grevillei*-2(strain EL 88289) were used in this study.Fresh sporocarps of these ectomycorrhizal fungi were collected at two mature forest stands in Northeast, China (Table 1). One stands of*Larixgmellini* located at Mohe County, Heilongjiang province. The other stand of *L. kaempferi* was at Kuandian County, Liaoning province.

Fungal pure culture

Mycelia of ectomycorrhizal fungi described above were each cultured in Petri dishes on a specificmedium with 1.8% agar, 10% potato juice, 50% wort, 2% glucose and 25mg/L vitamin$_{B1}$ at pH 6.5 and an incubation temperature of 25±1 °C. A gradient of pH including 2.5, 3.0, 4.0, 5.0, 5.5, 6.0, 7.0, 7.5, 8.0 and temperature at 10, 15, 20, 25, 28, 30, 35 and 40°C was designed to test the effect of pH and temperature on growth of EMM.After 7-28 days, depending on fungal species, mycelia were harvested and thefreeze-driedsamples were stored separately based on the methodological requirements of the procedures for fatty acidanalysis as described below.

Fatty acids analysis

The fatty acids of ECM myceliawere carried out with a four-step procedure according to KarliDskiet al.,[31] with minor modifications. Briefly, (1) saponification: each sample (40 mg DW) was added in 1.0 ml of saponification reagent (3.75MNaOH in methanol, 1:1, v/v) in a glass vial and placed in a 100 °C water bath for 5 min. The glass vial was vortexed vigorously for 10 s and returned to the water bath for additional 25 min. (2) methylation: after cooling, samples were added 2ml of methylation reagent (6N HCl in methanol, 3.25/ 2.75, v/v) and placed in a 80 °C water bath for 10 min. (3) Extraction: the fatty acid methyl esters were extracted by adding 1.25 ml extraction solvent (hexane+methyltert-butyl ether, 1:1, v/v) and mixing the vials for 5 min in a rotary shaker followed by

removal of the aqueous lower phase. (4) Wash: the resulting extract was washed with 3ml of base wash solution (0.3Maqueous NaOH) to remove nonmethylated fatty acids and other residues. The organic phase was then transferred to another vial for analysis.

Separation of FAMEs were performed using an Agilent GC (7890) equipped with a 30m fused silica capillary column (DB-5). The injector temperature was 280 °C and the detector temperature was 320 °C. The temperature was programmed at 140 °C for 3 min, raised from 140 °Cto 190 °C by a rate of 4°C min^{-1} and held constant at 190 °C for 1 min, then raised from 190 to 230 °C by a rate of 3°C min^{-1} and held constant at 230 °C for 1 min, then raised from 230 to 250 °Cby a rate of 2°C min^{-1} and held at 250 °C for 2 min and finally raised from 250 to300 °C by a rate of 10 °C min^{-1} and held constant at 300 °C for 1 min. The duration of the analysis was 48.83 min. One ¼l of sample preparation was injected. Thesoftware package Sherlock Version 6.2(MIDI Inc.) wasused for fatty acids identification.

Data analysis

The concentration (percentage) of fatty acids was used toanalyze the difference of signature fatty acids between species and community.Only fatty acids with over 0.5% relative abundance weresubjected to cluster analysis, principal component analysis (PCA) and nonmetric multidimensional scaling analysis (NMS), respectively. Thescores of the first two components from the PCA or NMS were usedto compare differences among the species of ectomycorrhizalfungi (larch and Norway spruce).All graphs were performed by Statistica7 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Effect of pH and temperature on EMM growth

In order to adapt host trees and environmental conditions, each species of ECM fungi could grow rapidly under optimal conditions, in particular, pH and temperature. Through pure culture, we compared the effect of pH and temperature on ECM mycelia (Table 1). All of species could grow in a wide variety of pH 4.0~8.0 and temperature of 10~30°C. However, both *Boleteuspictus* and *Suillus grevillei-2* grew under maximum optimal condition of pH 4.0 and a temperature of 20 °C, indicating that these two species developed well under acidic environment. In contrast, maximum optimal value of pH of *Cortinarius* sp. and *Lactariusisulsus* was 7.5, whose temperature was 25, 30°C, respectively (Table 1). Soil pH and temperature are determinants for fungal diversity, microbial community structure and biogeography[25,27], while ectomycorrhizal symbiosis can enhance host trees resistance to pH and temperature stresses[25-27]. In addition, temperature can exert an important effect on EMM growth of ECM fungi[28].These factors together with soil types may pay key roles in ECM fungal diversity and distribution[29]. Our results indicated that the ECM fungal species with wide range of pH and temperature could enable symbiont to fit performance under varying environment conditions.

Fatty acids profiles of ECM fungal species in larch

Themyceliafatty acids profiles of eight ECM fungal species were dominated by the fatty acids18:2É6,9c (16.26~59.89%),18:1É9c (7.46~34.48%), 16:0 (9.38~27.71%), and 10Me 18:0 TBSA (2.80~11.80%), accounting for up to 80% of relative abundance, while the total percentage of 18:2É6,9c, 18:1É9c and 16:0 in Norway spruce was up to 85%. while i16:0, 18:2É6,9t, 18:1É5c and i19:0 were in minor quantities (Table 2). The presence of 10Me 16:0 was the characteristic feature of all species tested. There were distinctive fatty acids profiles between *S. grevillei*-1 and *S. grevillei*-2. In particular, the relative abundance of 18:2É6,9c and 10Me 18:0 TBSA was less in *S. grevillei*-1 than *S. grevillei*-2 (Table 2).

The shifts in fatty acids composition among ECM fungal species were indicated by the two-dimensional ordination plots using NMS of fatty acids profiles (Figure 1). Within these plots, the closer the points, the more similar they are in fatty acid pattern. The species of *B. pictus*, *L.isulsus* and *S. grevillei*-1 had a dramatically different fatty acid composition from the other species, while the longest distance (2.614) occurred between *L.isulsus* and *S. aeruginascens*. Conversely, the distance (0.461) between *Cortinarius* sp. and *S. greillei*-2 was shorter than the distance between *Boleteus* sp. and *S. aruginascens* or *L. scabrum*, whose values were 0.468, 0.462, respectively (Figure 1). Particularly,

Table 1. Effect of pH and temperature on ECM fungal species in *Larixgmellini* and *L. kaempferi*

Species	LAT	MAT (°C)	MAP (mm)	Host tree	pH	pH range	Temperature (°C)	Temperature range(°C)
*Boleteus*sp.	N53°34' ,E124°18'	-5.5	400~460	*L. gmelini*	5.5	4.0~8.0	20	10~25
Boleteuspictus	N53°34' ,E124°18'	-5.5	400~460	*L. gmelini*	4.0	4.0~5.5	20	10~25
*Cortinarius*sp.	N53°34' ,E124°18'	-5.5	400~460	*L. gmelini*	7.5	2.5~8.0	25	10~30
Leccinumscabrum	N53°34' ,E124°18'	-5.5	400~460	*L. gmelini*	5.5	3.0~8.0	20	10~30
Lactariusisulsus	N53°34' ,E124°18'	-5.5	400~460	*L. gmelini*	7.5	3.0~8.0	30	10~30
Suillusaeruginascens	N53°34',E124°18'	-5.5	400~460	*L. gmelini*	6.5	4.0~8.0	28	10~35
Suillusgrevillei-1	N53°34' ,E124°18'	-5.5	400~460	*L. gmelini*	5.5	4.0~8.0	20	10~25
Suillusgrevillei-2	N40°43' ,E124°46'	6.5	1000~120	*L. kaempferi*	4.0	4.0~5.5	20	10~25

LAT: latitude; MAT: mean annual temperature; MAP: mean annual precipitation

Table 2. Fatty acids profiles from mycelia of ectomycorrhizal fungi in larch

Fatty acids	*Boleteus* sp.	*Boletinus pictus*	*Cortinarius* sp.	*Hebeloma masophaum*	*Lactarius* sp.	*Leccinum scabrum*	*Suillus aruginascens*	*Suillus grevillei*-1	*Suillus grevillei*-2
3OH10:0	3.05 (0.33)	ND	ND	ND	ND	ND	ND	ND	ND
12:0	7.77 (0.89)	ND	ND	ND	ND	ND	ND	ND	ND
2OH 12:0	ND	0.27 (0.02)	5.75 (0.08)	ND	1.82 (.021)	0.58 (0.04)	ND	1.36 (0.10)	0.39 (0.02)
14:0	ND	0.58 (0.03)	ND	ND	ND	1.02 (0.20)	ND	1.28 (0.10)	0.57 (0.02)
16:1ω5c	ND	0.82 (0.04)	ND	ND	ND	1.91 (0.22)	ND	1.44 (0.11)	0.84 (0.03)
16:0	16.13 (2.11)	26.12 (3.88)	17.07 (1.91)	13.16 (1.01)	19.26 (3.15)	18.24 (2.44)	9.38 (0.71)	27.71 (3.76)	26.35 (3.20)
10Me 16:0	4.06 (0.55)	4.62 (0.67)	3.77 (0.55)	3.27 (0.26)	3.80 (0.27)	3.51 (0.30)	3.82 (0.32)	4.80 (0.33)	4.77 (0.40)
18:2ω6,9c	37.04 (4.29)	16.65 (3.66)	52.11 (9.43)	59.89 (14.22)	38.24 (3.66)	30.91 (5.71)	47.93 (3.89)	21.32 (3.99)	16.26 (1.81)
18:1ω9c	18.22 (2.26)	33.36 (6.10)	7.46 (0.59)	4.08 (0.38)	21.70 (3.41)	23.40 (1.69)	19.80 (2.77)	27.44 (4.31)	34.48 (3.02)
18:0	6.57 (0.37)	7.30 (0.81)	2.16 (0.36)	5.44 (0.77)	2.98 (0.23)	8.14 (1.35)	5.56 (0.63)	5.86 (0.76)	6.45 (0.42)
10Me 18:0 TBSA	7.17 (0.35)	2.92 (0.04)	11.68 (2.34)	11.80 (1.52)	7.59 (0.56)	6.24 (1.08)	9.69 (0.85)	4.26 (0.71)	2.80 (0.17)
18:1ω9t	ND	5.73 (0.98)	ND	ND	4.61 (0.61)	4.49 (0.91)	3.82 (0.25)	4.53 (0.64)	5.89 (0.39)
18:1ω5c	ND	1.64 (0.03)	ND	2.36 (0.38)	ND	1.56 (0.14)	ND	ND	1.19 (0.20)

Data are presented as % relative abundance with means of 5 cultures and standard error (SE). SEwithin parentheses is for individual species

signature fatty acid of *S. grevillei*-1 was separated from that of *S. grevillei*-2. *S. grevillei*-1 originated from the host tree of *L. gmelini* grew in higher latitude, lower temperature and precipitation than *S. grevillei*-2 from host tree *L. kaempferi*(Table 1, Figure 1). Multivariate analysis may illustrate the effect of host trees on microbial community structure[30]. It is, however, relatively little known whether a nonmetric multidimensional scaling (NMS) could elucidate the difference among the pure culture mycelia of ECM fungal species. The current results clearly indicated a key role for host trees and such environment factors as temperature and precipitation in shaping fatty acids pattern of ECM fungal species in larch.

Comparison of fatty acids pattern of ECM fungal species in larch and in Norway spruce

To our knowledge, few studies concentrated on fatty acids profiles of ECM fungal species, especially for pure culture mycelia. A recent study showed that six species of ECM fungi in Norway spruce plantation located 3 different sites, including *Paxillusinvolutus*, *Amanita muscaria*, *Xerocomusbadius*, *Xerocomussubtomentosus*, *Suillusluteus* and *Suillus bovines*, were used to analyze fatty acids pattern by whole cell fatty acids composition[31]. On the basis of the data of ECM fungal species in Norway spruce and the data generated in this study, the chemical stoichiometry and principal component analysis were done. Subsequently, a significant difference was found in both specific fatty acids stoichiometry and the FA composition between larch and Norway spruce (Table 3; Figure 3). For example, the ratio of 18:1É9c to 16:0 was almost one-to-one in all the species but for the species of *Cortinarius* sp., *H.masophaum*, *S.aruginascens* in larch and the species of *A.muscaria*, *X.badius* and *X.subtomentosus*in Norway spruce, respectively. Interestedly,the ratio of 16:0-to-10Me 16:0 and 18:2ω6,9c-to-10Me 18:0 TBSA had almost the similar value of 5:1 with regard to ECM in larch (Table 3). Moreover, some fatty acids of low relative abundance in larch differed from the ones in Norway spruce. For example, the fatty acids of TBSA 10Me 18:0, 10Me 16:0 and 16:0 iso only were detected in larch(Table 2), while other fatty acids of 16:1ω11c, 2OH 16:0 and 17:1ω8c occurred in Norway spruce[31]. Similarly, PCA clearly distinguished fatty acids pattern of ECM fungal species between in larch and in Norway spruce (Figure2), each of which occupied very different ordination space. Composite scores for species in Norway spruce occupied the negative portion of the PC 1 (accounting for 29% of the variation), on which fungal species (*S. luteus*, *P. involutus*) received large negative weights (-1.050 and -2.451). In contrast, composite scores for the species in larch resided on the positive portion of PC 1, the high weights (0.479 and 2.436) given to the larch species of *S. aeruginascens* and *S. grevillei*-1. On PC 2 (accounting for 23% of the variation), the larch species of *S. grevillei*-1 and *Lacterius* sp. were separated from the others species due to high weights (5.024, -2.704), respectively (Figure2).

Although regular fatty acids presented in larch ECM mycelia, they possessed specific FA composition and stoichiometry in each species, implying that fatty acids profiles was species-specific and could be used as the basis of classification of ECM fungal species. Actually, FA profiles has been applied to comparisonof lipid contentdifference between Tuber fermentation mycelia and natural fruiting bodies[32],and identification of bacteria and yeast through the MIDI system[33,34]. Another observation of from these data was that host trees on fatty acids pattern of ECM fungal species was more effective than that of environmental variables. The species of Norway spruce cluster together was a clear discrimination in the ECM species cluster of larch (Figure 1&3). These results could be accounted for that both the factors of host trees such as root exudates and litters, and environment variables as soil pH and types might significantly change the ECM fungal community structure[35,36]. All the observation; however, would not support our expectation that we would like to determine the specific fatty acid marker to estimate the biomass, production and turnover of extrametrical mycelia of ECM fungi in forest ecosystems, where EMM plays a critical role in C cycling and mycorrhizalmycelial networks[28]. Neither fatty acid markers of 18:2ω6,9c and 18:1ω9c illustrated in this study, nor another biochemical marker of ergosterol was ECM specific[15,28,31]. Clearly, there are questions about biochemical marker of ECM fungi at the present studies and thus more extensive researches in order to fully elucidate the role of EMM in belowground carbon allocation and C turnover are warranted.

Table 3.Chemical stoichiometry of specificfatty acids from ectomycorrhizal fungalmycelia in larch and Norway spruce

Species	16:0/ 18:0	18:1ω9c/ 16:0	18:1ω9c/ 18:0	18:2ω,9c/ 16:0	18:2ω6,9c/ 18:0	18:2ω6,9c/ 18:1ω9c	16:0/18:2ω6,9c/ 18:1ω9c	16:0/10Me 16:0	18:2ω6,9c/10Me18:0 TBSA
ECM in larch									
*Boleteus*sp.	5:2	1:1	3:1	7:3	11:2	2:1	2:5:1	4:1	5:1
Boletinuspictus	7: 2	1: 1	4: 1	2: 3	2:1	1:2	3:2:4	5:1	5:1
*Cortinarius*sp.	8: 1	1: 2	4: 1	3: 1	24:1	7:1	2:6:1	5:1	5:1
Hebelomamasophaum	5: 2	1: 3	1: 1	9: 2	11:1	15:1	1:5:1	4:1	5:1
*Lactarius*sp.	13: 2	1: 1	7: 1	2: 1	13:1	3:1	1:2:1	5:1	5:1
Leccinumscabrum	7: 3	1: 1	3: 1	3: 2	4:1	1:1	2:4:3	5:1	5:1
Suillusaruginascens	7: 4	2: 1	4: 1	5: 1	17:2	5:2	1:5:2	3:1	5:1
Suillus grevillei-1	5: 1	1: 1	4: 1	1: 1	9:2	1:1	4:3:4	5:1	5:1
Suillus grevillei-2	4: 1	1: 1	5: 1	2: 3	5:2	1:2	3:2:4	5:1	5:1
ECM in Norway spruce									
Amanita muscaria	3: 2	3: 1	4: 1	8: 1	12:1	3:1	1:8:3	-	-
Paxillusinvolutus	15: 1	1: 1	16: 1	3: 1	23:1	3:1	1:3:1	-	-
Suillusluteus	18: 1	1: 1	20: 1	7: 1	14:1	6:1	1:7:1	-	-
Suillus bovines	7: 1	1: 1	8: 1	5: 1	43: 1	6:1	1:6:1	-	-
Xerocomusbadius	18: 1	1: 3	6: 1	4: 1	63: 1	10:1	3:10:1	-	-
Xerocomussubtomentosus	33: 1	1: 4	8: 1	3: 1	98: 1	12:1	4:12:1	-	-

The data in Norway spruce were collected from the literature.[31]

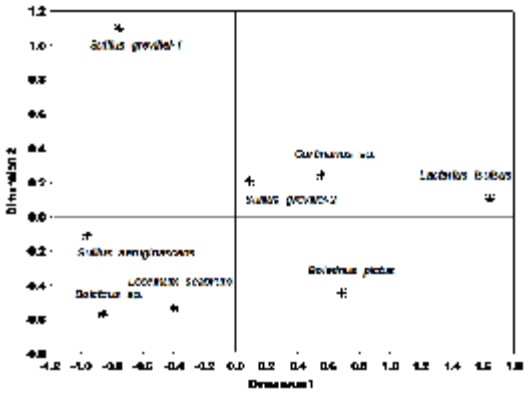

Fig. 1. Multidimensional scaling analysis of PLFA concentrations (% relative abundance) among different species of ectomycorrhizal fungi in larch. (Stress: 0.0001)

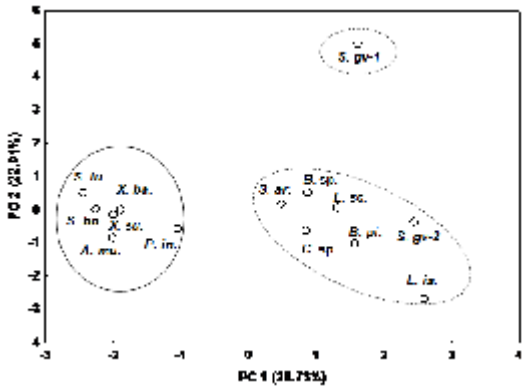

Fig. 2. Fatty acids pattern of hyphae pure cultures of ectomycorrhizal fungi representing six fungal species in Norway spruce (*P. in.*:*Paxillusinvolutus*, *A. mu.*: *Amanita muscaria*, *X. ba.*: *Xerocomusbadius*, *X. su.*: *Xerocomussubtomentosus*, *S. lu.*:*Suillusluteus*, *S. bo.*: *Suillus bovines*) and eight species in larch *(B.* sp.: *Boleteus*sp., *B. pi.*: *Boletinuspictus*, *C.* sp.: *Cortinarius*sp., *L. sc.*: *Leccinumscabrum*, *L. is.*: *Lactariusisulsus.*, *S. ar.*: *Suillusaeruginascens*, *S. gv-1*: *Suillus grevillei-1*, *S. gv-2*: *Suillus grevillei-2*).The data in Norway spruce were collected from the literature.[31]

CONCLUSION

In this study, we measured the effect of pH and temperature on growth of seven ECM mycelia from *L. gmelini*and one from *L. kaempferi*,and showed that only *B. pictus* and *S. grevillei*-2 grew under a narrow range of pH (4.0~5.5) and temperature (10~25 °C), while the others species could grow under wide range of pH, from 4.0~8.0, and at 10~30°C, indicating each species with different the maximum optimal growth condition. Furthermore, we determined fatty acids fingerprinting of the ECM species in larch and then compared them with published data in Norway spruce using principal components analysis and multidimensional scale analysis. We found that the four main fatty acids, 18:2ω6,9c, 18:1ω9c, 16:0and TBSA 10Me 18:0 ECM mycelia in larch, accounted for 80% of relative abundance. Not species-specific fatty acids biomarker but major fatty acids stoichiometry differed among the ECM mycelia.The ratio of 18:1ω9c / 16:0 was 1: 1 in all the ECM mycelia, and the ratio of 16:0/10Me 16:0 and 18:2ω6,9c/TBSA 10Me 18:0 were 5: 1 in limit to ECM mycelia of larch.Both fatty acidspattern and FA stoichiometry was also clear difference between*S. grevillei*-1 in *L. gmelini*and *S. grevillei*-2 in *L. kaempferi*.Taken together, these findings suggested that fatty acids profiles of pure cultured mycelia might have a chemotaxonomic potential in ECM fungal species, and that FA composition as well as FA stoichiometry were affected by the host tree species and environmental factors.

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