山地林と都市近郊林における樹木の葉内生菌相の比較

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キーワード；山地林、都市近郊林、宿主特異性、水平伝播、内生菌群集

1. はじめに

植物内生菌（endophytic fungi　以下内生菌）とは、生活環のある時期において、病徴を現すことなく、生きた宿主植物の組織内に生息する菌類のことであり、それらと宿主の相互関係について研究がなされてきた。樹木内生菌の機能としては、潜伏性病原菌、潜伏性分解菌、病原菌や害虫に拮抗する共生菌といった多様なふるまいが報告されている。樹木内生菌は胞子により水平伝播するため、内生菌の群集組成は、気温、湿度、降水量などの非生物的環境要因、宿主の分類学的な系統、林冠密度、植生、植食者との関係などの生物的環境要因によって影響を受ける。内生菌には宿主特異性の強いものと宿主範囲の広いものとがあり、それらの割合は、周辺植生などの林内環境や森林群落の歴史を反映し変化することが予想される。本研究では、1）様々な樹種の内生菌相の調査を行うことにより、内生菌の宿主範囲を検討し、2）山地の森林と都市化した孤立林の樹木内生菌相の比較により、環境が内生菌相に与える影響を検討した。

2. 調査地および調査方法

高尾山･吾国山の山地林と千葉県柏市内の孤立した都市近郊林を調査地とし､2006､2007 年の夏季に各地で 3 回ずつ調査を行った｡計 10 樹種（全調査地共通: シラカシ､ヒサカキ、スギ､ヒノキ ； 高尾山:コナラ､イヌブナ､アラカシ ； 吾国山: ミズナラ､イヌシデ; 柏: コナラ、クリ）を対象に、各樹種 3 個体から外見上健全な成葉を 15 枚ずつ採集した。採集したサンプルを流水洗浄後、エタノール・アンチホルミン系で表面殺菌し、広葉樹の葉は 2 部位から直径 6mm のディスクを、スギでは長さ 15mm の針葉、ヒノキでは 5mm の一対の鱗片葉を含む一節を、1 試料とし切り出した。それらを改変 1/2PDA 培地、20℃暗黒条件下で培養した。分離率（IF）は、**IF(%)＝(各分類群が出現した葉片数／供試片数)×100** で算出し、相対優占度（RD）は、$RD\ (\%) = IF_i / \Sigma\ IF_i \times 100$ で算出し、RD が 1%に満たない菌群は、“rare fungi”とした。相対優占度から優占菌を判定し、菌株数や菌群数の比較には Tukey-Kramer’s HSD を、群集組成の比較にはクラスター解析を用いた。

3. 結果および考察

3 調査地で 4320 サンプルを供試し、計 6071 菌株を分離した。吾国山で 39 菌群、高尾山で 40 菌群、柏で 38 菌群、あわせて 11 属 16 種を含む 44 菌群が出現した。宿主あたりの出現菌群数の範囲は、吾国山で 13～24 菌群、高尾山で 15～25 菌群、柏で 14～28 菌群で調査地間の差はなかった。既往研究でも多くの樹種に出現が報告されている *Colletotrichum* 属、*Phomopsis* 属および *Phyllosticta* 属は全ての宿主樹種で観察された。調査地が異なっても、同樹種では多くの優占菌が共通した。また優占菌は、出現した樹種数により宿主選好性の高い菌、低い菌、それ以外の菌に分けられた（表 1)。シラカシ、コナラなどでは、山地林で宿主選好性の高い菌が多く、柏では宿主選好性の低い菌が増加した。クラスター解析の結果（図 1)、内生菌群集はナラ類とカシ類がそれぞれクラスターを

形成し、他も多くは樹種ごとにまとまった。したがって、優占菌だけでなく内生菌群集全体の類似性が宿主の分類学的な系統を反映していることが示唆された。一方、他地点の同樹種よりも同調査地の他樹種と類似したものもあったことから、周辺植生が内生菌相へ影響することが示された。

表 1. スギ、シラカシ、コナラおよびミズナラの内生菌相（分離率; %）

	宿主	スギ			シラカシ			ミズナラ	コナラ	
宿主選好性*	出現菌群 / 調査地	吾国	高尾	柏	吾国	高尾	柏	吾国	高尾	柏
	Phyllosticta sp.	57.0	74.8	57.0	0.7	7.8	14.8		5.6	11.5
	Phomopsis sp.1	5.9	3.7	1.5	5.6	3.0	13.3	33.7	23.7	34.8
	Colletotrichum gloeosporioides	0.7		9.6	8.1	7.0	6.7		9.6	11.1
	Colletotrichum acutatum				3.7	1.5	0.4	1.1	3.3	1.5
	Glomerella sp. (*Colletotrichum* sp.3)	1.5	0.7		5.6	5.6	4.1	0.7	10.7	2.6
低	White sterile WH5	32.6	0.7		3.0	0.4		0.7	0.7	
	White sterile WH6	43.7	3.0	9.6		0.4	2.2	0.7	4.4	4.8
	White sterile WH7	6.7		1.5	1.5	0.4	5.2	1.5	0.4	3.0
	White sterile WSW	0.7		2.2	18.5	5.2		2.2	1.9	0.7
	White sterile WM1			4.4	0.4		1.9	6.3		8.9
	White sterile PS1	2.2	10.4		0.7	0.4	1.5	6.7	7.8	1.9
	QA-b				15.6	47.4	1.1		0.4	0.7
	Dark sterile QMG				14.8	33.0	12.2			
高	*Discula* sp.3				1.5	10.7	1.5			
	Discula sp.1	0.7				1.9	0.7	62.6	58.5	27.0
	Red sterile WM34			0.7				11.1		0.4
	その他の菌群	6.7	8.9	8.9	4.4	5.9	6.7	8.1	9.3	23.0
	Rare fungi	4.4	3.0	2.2	4.4	0.7	1.5	8.5	3.3	1.1
総分離率（%）		94.8	89.6	81.5	73.3	83.0	65.6	95.6	95.6	93.0
出現菌群数		13	15	14	20	22	27	24	20	28

*優占菌のみを表示した。 ■：優占種 □：第一位優占種
*宿主選好性の低い菌群；相対優占度が1%以上を示した樹種数が4以上：高い菌群；1%以上の樹種数が1~3かつ4%以上が1以上

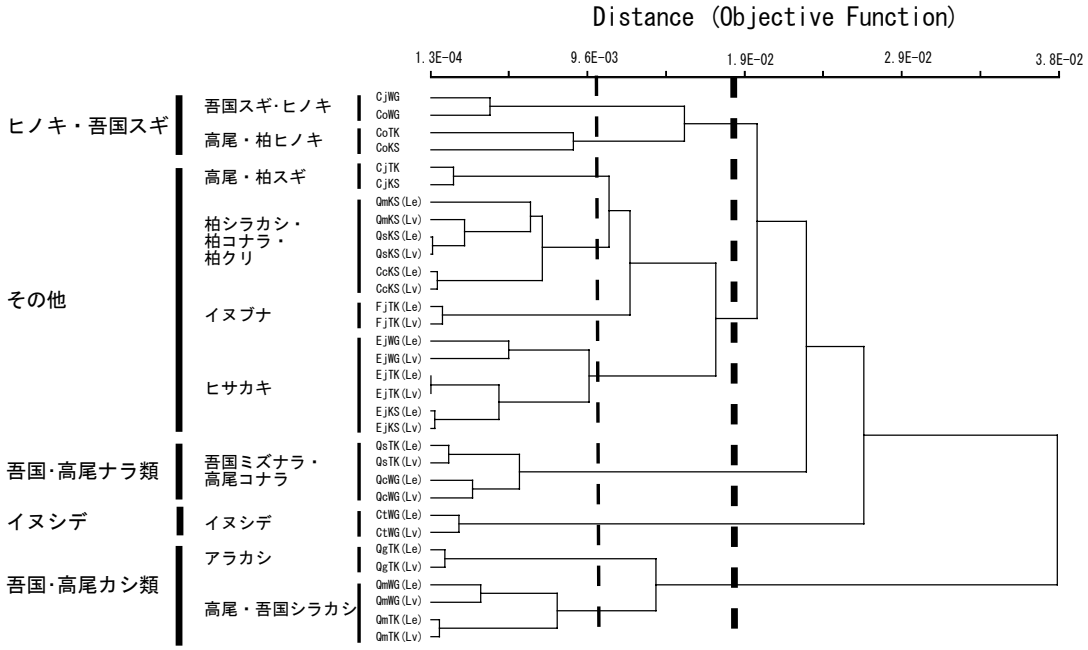

図 1. クラスター解析結果

*Qm: シラカシ, Ej: ヒサカキ, Cj: スギ, Co: ヒノキ Qs: コナラ, Fj: イヌブナ, Qg: アラカシ, Qc: ミズナラ, Ct: イヌシデ, Cc: クリ

*WG: 吾国, TK: 高尾, KS: 柏 *Lv: 主脈を含む葉部, Le: 葉縁を含む葉部

Comparison of endophytic fungal assemblages in tree leaves of rural and suburban forests

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Key words; rural forest, suburban forest, host specificity, horizontally transmitted, endophytic assemblage

1. Introduction

Plant endophytic fungi (endophytes) is defined as all fungal species that live in plant tissue without producing any external symptoms at some time in their life cycle, and their interactions with hosts have been studied. Various functions of endophytes are reported such as pathogen, decomposer, and symbiont. As tree endophytes are horizontally transmitted by fungal spores, the composition of endophytic assemblage in a tree leaf is influenced either by abiotic environmental factors, i.e. temperature, humidity, rainfall, or biotic environmental factors, i.e. the host phenotype and genotype, vegetation, canopy density, and the herbivores acting as vectors of the endophytes. Some endophytes are host-specific and others have wide host-range, and those proportions may change by environmental factors. The aims of this study are: 1) to investigate the endophytic assemblages of various tree species to clarify host-range of endophytes, and 2) to examine the difference in endophyte assemblage between rural forests and an isolated suburban forest.

2. Materials and Methods

The study sites were Mt. Wagakuni and Mt. Takao (Rural forests), and Kashiwa (Suburban forest). The isolation of endophytes was conducted 3 times per sites during the summer in 2006, 2007. A total of 15 symptomless leaves were collected from three trees of each of tree species (All sites: *Quercus myrsinaefolia*, *Eurya japonica*, *Cryptomeria japonica, Chamaecyparis obtusa*; Takao: *Quercus serrata, Fagus japonica*, *Quercus glauca*; Wagakuni: *Quercus crispula*, *Carpinus tschonoskii*; Kashiwa: *Quercus serrata, Castanea crenata*). The collected samples were washed under running tap water, and then surface sterilized with ethanol solution and NaClO. Two leaf disks from a broadleaf with a diameter of 6mm were punched out, a *Cryptomeria* needle was cut into 15mm length, and a pair of *Chamaecyparis* scales was cut out. The samples were incubated on modified 1/ 2 PDA in a Petri dish at 20℃ in the dark. The isolation frequency (IF) of a single endophyte taxon was calculated as: IF (%) = (the number of segments from which the fungus was isolated) / (the total number of segments cultured) × 100. The relative dominance (RD) of a fungus was calculated as: $RD\ (\%) = IF_i / \Sigma\ IF_i \times 100$. Fungal taxa whose RD was less than 1% were regarded as “rare fungi”. The dominant fungi were decided by RD. Tukey-Kramer HSD was used to examine the differences in the isolation frequency, and cluster analysis was used to compare endophytic composition.

3. Results and Discussion

A total of 4320 sample from all tree, from all three sites were processed, and 6071

isolates were recovered. A total of 44 taxa including 16 species belonging to 11 genera were isolated: 39 taxa in Wagakuni, 40 taxa in Takao and 38 taxa in Kashiwa, respectively. The number of fungal taxa ranged 13 to 24 in Wagakuni, 15 to 25 in Takao and 14 to 28 in Kashiwa, and there were no significant difference between sites. Species of *Colletotrichum, Phomopsis* and *Phyllosticta,* reported to have wide host-range were observed in all tree species. Regardless of sites, same host species shared many common dominant fungi. The dominants were divided by host preference into highly host-selective, wide-range, and others (Table 1). In *Q. myrsinaefolia* and *Q. serrata,* highly host selective fungi were dominant in rural forests, while wide-range fungi were dominant in Kashiwa. Result of cluster analysis (Fig. 1) showed that endophytic assemblage was grouped by host species and sub-genus. Therefore, it is suggested that endophytic assemblage reflects phylogeny of host plants. On the other hand, some different host species from the same site were grouped in some cases, i.e. surrounding vegetation also influences endophytic assemblage.

Table 1. The endophytic assemblages (The isolation frequency: %) of *C. japonica*, *Q. myrsinaefolia*, *Q. crispula*, *Q. serrata*

	Host site	*C. japonica*			*Q. myrsinaefolia*			*Q. crispula*	*Q. serrata*	
Host selectivity	Fungal taxa	WaGakuni	Takao	Kashiwa	WaGakuni	Takao	Kashiwa	WaGakuni	Takao	Kashiwa
Wide-range	*Phyllosticta* sp.	57.0	74.8	57.0	0.7	7.8	14.8		5.6	11.5
	Phomopsis sp.1	5.9	3.7	1.5	5.6	3.0	13.3	33.7	23.7	34.8
	Colletotrichum gloeosporioides	0.7		9.6	8.1	7.0	6.7		9.6	11.1
	Colletotrichum acutatum				3.7	1.5	0.4	1.1	3.3	1.5
	Glomerella sp. (*Colletotrichum* sp.3)	1.5	0.7		5.6	5.6	4.1	0.7	10.7	2.6
	White sterile WH5	32.6	0.7		3.0	0.4		0.7	0.7	
	White sterile WH6	43.7	3.0	9.6		0.4	2.2	0.7	4.4	4.8
	White sterile WH7	6.7		1.5	1.5	0.4	5.2	1.5	0.4	3.0
	White sterile WSW	0.7		2.2	18.5	5.2		2.2	1.9	0.7
	White sterile WM1			4.4	0.4		1.9	6.3		8.9
	White sterile PS1	2.2	10.4		0.7	0.4	1.5	6.7	7.8	1.9
Host-selective	QA-b				15.6	47.4	1.1		0.4	0.7
	Dark sterile QMG				14.8	33.0	12.2			
	Discula sp.3				1.5	10.7	1.5			
	Discula sp.1	0.7				1.9	0.7	62.6	58.5	27.0
	Red sterile WM34			0.7				11.1		0.4
Others	Other fungi	6.7	8.9	8.9	4.4	5.9	6.7	8.1	9.3	23.0
	Rare fungi	4.4	3.0	2.2	4.4	0.7	1.5	8.5	3.3	1.1
Proportion of total observed infection(%)		94.8	89.6	81.5	73.3	83.0	65.6	95.6	95.6	93.0
Number of fungal taxa		13	15	14	20	22	27	24	20	28

*Only dominants were showed. ■ : dominants □ : 1st dominant

* Wide range ; 4 ≦ Number of tree species (RD ≧1%)

Host-selective taxa ; 1 ≦ Number of tree species (RD ≧1%) ≦3, and Number of tree species (RD ≧ 4%) ≧1

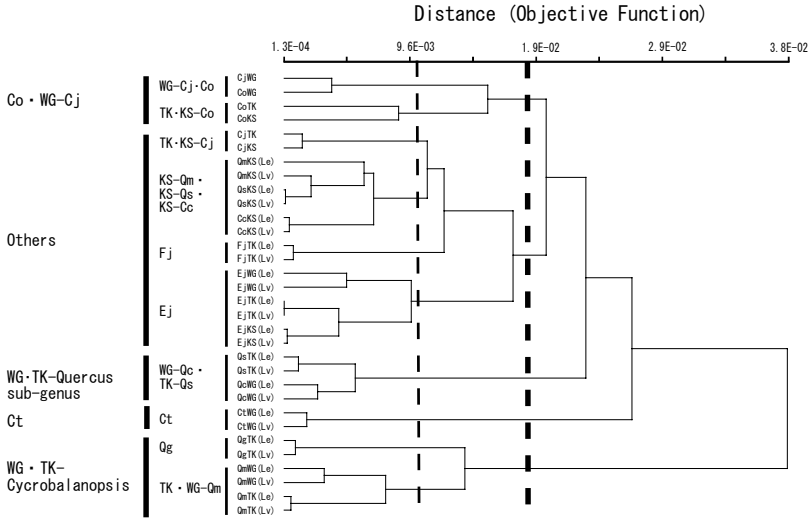

Fig. 1. The result of cluster analysis

*Qm: *Q. myrsinaefolia*, Ej: *E. japonica,* Cj: *C. japonica*, Co: *C. obtusa*,Qs: *Q. serrata,* Fj: *F. japonica*, Qg: *Q. glauca,* Qc: *Q. crispula*, Ct: *C. tschonoskii*, Cc: *C. crenata*

*WG: Wagakuni; TK: Takao; KS: Kashiwa *Lv: leaf vein, Le: leaf edge