The Occurrence and Distribution of Cellular Slime Molds in Major Deciduous Forests of South Korea

Hong, Jeong-Soo and Nam-Kee Chang

(Department of Biology Education, Scoul National University)

南韓의 主要 落葉樹林에서 細胞性 粘菌의 出現 및 分布

洪 廷 守・張 楠 基 (서울大學校 師範大學 生物教育科)

ABSTRACT

The occurrence and distribution of cellular slime molds in major deciduous forests of South Korea were investigated. Ten species were isolated from the soils collected at ten sampling areas and cultured in hay infusion agar with *E. coli*. These include: *Dictyostelium minutum* Raper, *Dictyostelium mucoroides* Brefeld, *Dictyostelium lacteum* Van Tiegham, *Dictyostelium polycephalum* Raper, *Dictyostelium fasciculutum* Traub, Hohl and Cavender, *Dictyostelium sp. Polysphondylium pallidum* Olive, *Polysphondylium violaceum* Brefeld, *Copromyxellar* sp. and *Guttulinopsis vulgaris* Olives. Among these species, *Dictyostelium* sp. and *Copromyxellar* sp. could not be identified. *D. minutum* and *D. mucoroides* occurred the most frequently in almost all the deciduous forests investigated in this study. *Dictyostelium* sp., *D. fasciculutum* and *Copromyxella* sp. occurred only in the north of the central region, but *D. lucteum* and *D. polycephalum* were found only in the south of that region. This indicates that distribution of these species may be affected by climate. The number of isolates per sampling site ranged from 2 to 6, averaging 4.3.

INTRODUCTION

Cellular slime molds are very small microorganisms which grow rapidly and can not be easily seen in the fields (Yamada, 1982). Because of their unusual life cycles including differential process of animal and vegatative stage or differentiation from unicellular form, cellular slime molds have been used for developmental, genetic and ecological studies.

Since Brefeld (1869) discovered *Dictyostelium mucoroides*, studies on cellular slime molds have been published and their taxonomical niche has been discussed (Traub and Hohl, 1976; Olive, 1975; Yamada, 1978; Maeda and Maeda, 1978; Youasa, 1982). The

name of cellula slime molds does not designate specific taxonomical one (Yamada, 1971, 1978). It was suggested that cellular slime molds generally include both the subclass Dityostelia and the subclass Acrasea within the class Mycetozoa of Protista (Olive, 1975).

Cellular slime molds are widely distributed in nature (Cavender, 1973). They are believed to be distributed differently according to environmental conditions (Raper, 1956, Davender, 1969a, b, 1972, 1973, 1976b, 1980; Cavender and Raper, 1965c, 1968; Smith and Keeling, 1968; Whittingham and Raper, 1957; Benson and Mahoney, 1977). The occurrence and distribution of these organisms have been investigated in many countries and new species have been continually

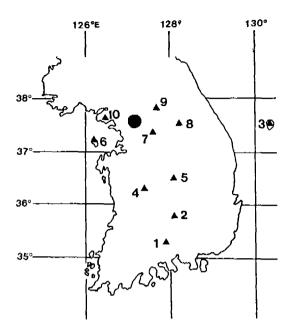


Fig. 1. Map of sampling area. The numbers refer to the collection sites described in Table 1.

reported (Olive, 1965; Nelson et al., 1967; Anderson et al., 1968; Cavender, 1976a, 1977; Raper and Kurzynski, 1978; Cavender and Norberg, 1979; Traub et al., 1981a,b). However there is no report on the occurrence and distribution of cellular slime molds in forests of South Korea. The purpose of this study is (1) to investigate the occurrence of cellular slime molds in deciduous forests of Korea, and (2) to identify and describe these organisms isolated from the samples, and (3) to investigate the their distribution.

MATERIALS AND METHODS

Sampling. Soil collection was carried out according to the procedure used by Cavender and Raper (1965, 1965b) and Benson and Mahoney (1977). Sampling areas were limited with eight major mountains and two islands (Fig. 1). Ten samples were collected randomly at the soil surface or from the humus layer of deciduous forests at each site (Table 1). Samples were transported to laboratory, and refrigerated at 5°C. Normally, samples were processed the next day.

Isolation. *Escherichia coli* was cultured in liquid medium, as a nutrient for cellular slime molds. The technique of isolation involved the used of a hay infusion

Table 1. Name, altitude, and forest types of 10 sampling sites

Number	Name	Symbol	Altitude(m)	Туре		
1	Mt. Chiri	CR	650	Oak Forest		
2	Mt. Dugyou	DY	620	Oak Forest		
3	Isnd. Ulleung	UL	500	Oak Forest		
4	Mt. Keryong	KR	550	Oak Forest		
5	Mt. Songli	SL	600	Oak Forest		
6	Isnd. Dugjuk	DJ	300	Oak Forest		
7	Mt. Namhan	NH	520	Mixed Forest		
8	Mt. Yongmoon	YM	600	Mixed Forest		
9	Mt. Chunma	CM	600	Oak Forest		
10	Mt. Mani	MN	400	Oak Froest		

agar plates infused from 8g leached, dried hay, 1.5g Na₂H PO₄·12H₂O and 5g agar/liter (Cavender and Raoerm 1865a; Kuserk *et al.*, 1977). Petri dishes containing hay infusion agar to a depth of 4-5 mm were prepared. Ten grams of soils were added into sterile distilled water to make 100 ml of suspension, giving an initial dilution of 1:10. Flasks were shaken for 2 min to break up soil particles and to distribute spores and myxamoeba. A dilution of 1:25 was made by adding 5 ml of the above suspension to 7.5 ml sterile water, and 0.5 ml of this dilution was added per plate, giving final dilution of 1/50g soil/plate. At the same time, 0.5 ml of a heavy suspension of a pregrown *E. coli* was deposited to provide nutrient or cellular slime molds. Five plates were prepared for each sample, incubated at 23 °C.

On the fourth and the sixth days of incubation the cellular slime molds were counted and isolated and cultured purely for furthr observations. Pure isolates were obtained by transferring spores from sori to fresh streaks of *E. coli* on 0.1% LP agar consisting of 1g lactose, 1g peptone, 15g agar and 1 liter of distilled water.

Identification. The method of idetifying and analyzing Acrasian populations was based on the key to cellular slime molds reported by Yamada (1982), Olive (1965, 1975), Raper and Fennel (1967), Raper and Worley (1977), and Traub *et al.* (1981b). Possibilities of variant in specific isolates were investigated according to previous studies (Huffman and Olive, 1963; Reinhardt, 1975). During the life cycles of isolates, the characteristics of their forms and length of spores, sorocarps, bases, sorophores, and aggregation and migration were observed and compared with one another in their differentiation process.

D. giganteum

8. Sorophores 1.7-2.5 cm

5. Migrates not leaving stalk at the surface

9. Migrates naked-snail in shape; soro-

of agar

carps solitary

The following values were calculated for each site: sample frequency (the number of samples containing clones divided by the total number of samples, times 100), site frequency (the number of sites containing clones divided by the total number of sites, times 100). A key to the Acrasiales is presented below.

rapidly

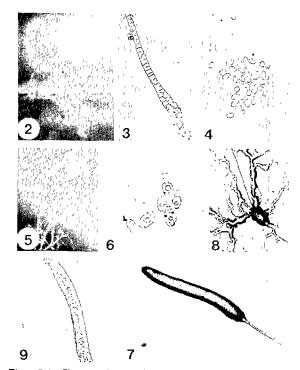
8. Sorophores less than 1.5 cm

......D. mucoroides

Dictyosteliales	A key to the Acrasiales is presented below.			10. Sorophores arising from disk-like
Dictyosteliales A. family Acytosteliaceae: Sorophores thin and uncellular. B. family Dictyosteliales: Sorophores thick. 1. Sorocarps not branched, or irregularly branced. Dictyostelium 2. Sorocarps regularly and verticillatedly branched Polysphondylium Acrasea A. family Acrasidae: Stalk cells and spore cells different in fors. Acrasea B. family Copromyxidase: Stalk cells and spore cells different in forms; sorocarps tree-shaped. C. family Guttulinopsidae: Stalk cell and spore cells identical in forms; sorocarps tree-shaped. C. family Guttulinopsidae: Stalk cell and spore cells identical in forms; sorocarps head-shaped Mentical in forms; sorocarps head-shaped C. family Guttulinopsidae: Stalk cell and spore cells identical in forms; sorocarps head-shaped Mentical in forms; sorocarps head-shaped A. Acytostelium A. Acytostelium 1. Spores uniform globular A. Acytostelium 1. Spores uniform globular A. Irregularosporum 1. Spores emiform globular A. Irregularosporum 1. Spores emiform globular A. Irregularosporum 1. Spores emiform globular A. Irregularosporum 1. Spores gliptical A. Cilipticum D. Dictyostelium 2. Sorocarps small (degree of 1 mm) in length: no-stalked sori attaching to sorophore D. Roscrium 1. Spores emiform globular A. Cilipticum D. Sorocarps small (degree of 1 mm) in length: no-stalked sori attaching to sorophore D. Roscrium D. Roscrium D. Coernivo-stipes 16. Sori grayish, lavender; sorophoers comparatively long, thin, purple or dark smoke shades 16. Sori deep vinaceous gray to dark 4. No migration stage 11. Sorophores moderately thick; sorocarps tree shaped 12. Sorophores moderately thick; sorocarps tree shaped 12. Sorophores few branched; spores small usually less thant 75 um 12. Sorophores few branched; spores ingelliptical D. Acmisis Sorophores few branched; spores to light gray hyaline. D. polycarphin 13. Sori only at the top 14. Sori white cream, yellowish, grayish or brownish; sorophores comparatively heavy, often long, frequently clustered or dark smok		Key to the families and the genera		 Sorophores not arising from disk-
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6. Sori milk-white or yellowish in color 1. Sori purple in color			C.	
7. Sorocarps irregular, often growing 1. Sori white-hyaline or milk-white in color				
rapidly				
D. trregularis D. Copromyxella			D.	
7. Sorocarps uniform, not growing 1. Sorocarps consisting of simple or sparsely branch-				

ed columns

 Clusters growing well and fruiting optimally in association with E. coli on appropriate



Figs. 2-4. Characteristics of D. minutum, Fig. 2. Fruiting bodies. \times 40, Fig. 3. Sorophore consists of a tier of cells except basal structure. \times 200, Fig. 4 Spores. \times 400. Figs. 5-9. Characteristics of D. mucoroides, Fig. 5 Fruiting bodies. \times 10, Fig. 6 Spores. \times 1000, Fig. 7 Migrate leaves stalk at the surface of agar. \times 100, Fig. 8 Aggregating st-

reaming. $\times 40$, Fig. 9 Thick sorophore. $\times 200$.

substrata, microcysts produced abundantly.

- Sorocarps developing singly and in close proximity, very delicate and fragile
- 2. Clusters not fruiting well in association with *E. coli* on any substrata; sorocarps often robust,
- - 4. Sorocarps coral-like in appearance; fruiting only with and upon indigenous bacteria

E Guttulinopsis

RESULTS

Occurrence and description. Ten species of cellular slime molds, 8 belonging to the family Dictyosteliaceae

and 1 to the family Guttulinopsidae and 1 probably to the family Copromyxidase, were isolated from the sampling soils. These are Dictyostelium minutum, Dictyostelium mucoroides, Dictyostelium lacteum, Dictyostelium polycephalum, Dictyostelium, Dictyostelium sp., Polysphodylium violaceum, Polyshondylium pallidum, Copromyxella sp. and Guttulinopsis vulgaris. Among these, Dictyostelium sp. and Copromyxella could not be identified yet. These seemed to be new species. Brief description of the characteristics of isolates are presented below.

Dictyostelium minutum Raper. Sorocarps clustered or commonly solitary, branced with variable size and spacing (Fig. 2). The sorocarps of D. minutum are easily recognized by their small size (200-925 μ m in height), and erect or semierect. Sorophore white-hyaline or semihyaline, arising from club-like based, commonly consiting of a single tier of cells throughout or except in basal area (Fig. 3). Sori not hyaline, globose, $30\text{-}140\,\mu\text{m}$ in diameter. Spores colorless hyaline, elliptical, $5.0\text{-}8.0\times3.0\text{-}4.2\,\mu\text{m}$ in size (Fig. 4).

Dictyostelium mucoroides Brefeld. Sorophores of this species are moderately thick (Fig. 9), varying from 3.5 to 20 mm in length, commonly solitary. Sori intermediate in size, commonly 70-160 μ m in diameter (Fig. 5). Spores thick elliptical 15-7×2-3 μ m (Fig. 6). Migrates distinctive, leaving stalks at the surface of agar (Fig. 7). Aggregation streaming distinctive (Fig. 8).

Dictyostelium lacteum van Tiegham. Sorocarps small, with very slender stalks, no branced, commonly clustered or solitary. Sori milk-white or yellowish. It is easily distiguished by the relatively small spherical spores (Fig. 10).

Dictyostelium polycephalum Raper. Sorocarps small, less than 1 mm in height. Sorophore formation initiated immediately in the area beneath each of conspicuous papillae, the mass of cells vertically divided into a number of separate but essentially parallel fractions or sorogens. Sorophores distinctly divided at the top (Fig. 11). Spores elliptical, $3-4 \times 2-3 \mu m$ in size.

Dictyostelium fasciculutum Traub, Hohl and Cavender. Sorocarps typically clustered or solitary (Fig. 15), mostly 2-4 mm. Streaming not distinctive. Sori globose, milk-wite to distinctively yellowish 30-60 μ m, almost 100-250 μ m. Sorophores colorless, occasionally branched, initiated from papillae (Fig. 12, 13), Spores oval to elliptical with characteristic polar or subpolar granules,

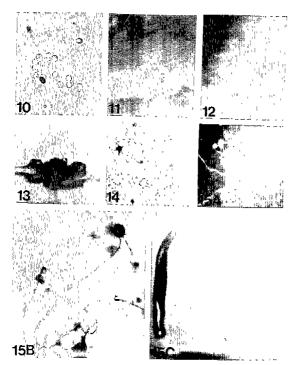


Fig. 10. Spherical spores of D. lacteum. $\times 400$, Fig. 11. Fruiting body of D. polycephalum. $\times 30$

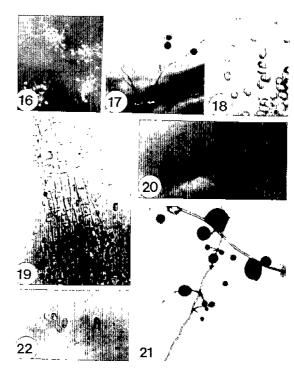
Figs. 12-15C. Characteristics of *D. fasciculutum*, Fig. 12 Papillae-like aggregation. ×30, Fig. 13 Lateral view of aggregation. ×40, Fig. 14 Spores. ×400, Fig. 15A Clustered fruiting bodies. ×15, Fig. 15B Lateral view of Fruiting bodies. ×40, Fig. 15C Solitary appearance resembles *D. mucoroides*. ×100.

$2-4\times4-8\,\mu\text{m}$ (Fig. 14).

Dictyostelium sp. Sorocarps small, delicate, typically clustered (Fig. 16), or solitary, occasionally branched (Fig. 17), generally range from 0.8 to 2 mm in length. Sorophore colorless or gray-hyaline, consisting of a tier of cells throughout except basal structure. Spores elliptical, $2\text{-}4\times4\text{-}8\,\mu\text{m}$.

Polysphodylium violaceum Brefeld. Sorocarps errect or semierrect, verticillatedly branched (Fig. 21). Sorophores thick, light purple in color, arising from club-like bases, 1.8-15.7 mm in length. Sori purple in color, 80-325 μ m in diameter, 35-175 μ m, in branches. colorless, elliptical, 5.6-8.4 × 2.8-5.0 μ m (Fig. 22). Myxamoeba streaming distinctive and pseudoplasmodium formed.

Polysphondylium pallidum Olive. Sorocarps commonly erect or semierect, having regularly verticillated branches (Fig. 23). Sorophores white, 1.2-13 mm in

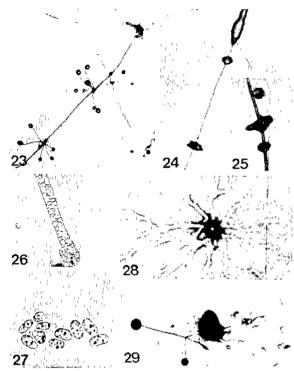


Figs. 16-20. *Dictyostelium* sp. Fig. 16 Clustered fruiting bodies. ×10, Fig. 17 Solitary appearance of fruiting bodies. ×100, Fig. 18 Spores. ×400, Fig. 19 Coremiform of stalk, ×100, Fig. 20 Initiation of stalk, ×100, Fig. 21-22 *P. violaceum*, Fig. 21 Fruiting body is purple in color. ×40, Fig. 22 Spores. ×40.

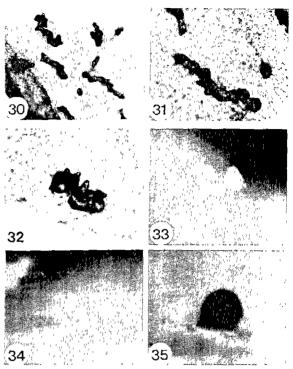
length, arising from club-like bases (Fig. 26). Sori or branches formed at the same time the stalk differentiated (Fig. 24, 25). Sori white-hyaline or milk-white, $45-130\,\mu\text{m}$ at the top, $20-72\,\mu\text{m}$ in the branches. Spores colorless, hyaline, elliptical, $5.8-8.0\times3.2-5.0\,$ m in size (Fig. 27).

Copromyxella sp. Fruiting structures commonly irregular-shaped (Fig. 30, 31), often profusely branched (Fig. 32), yellowish in color. Branches variable in length, pattern and diameter. Small colonies often aggregating and forming a large fruiting structure.

Guttulinopsis vulgaris Olive. Sorocarps typically short, head-shaped, 95-465 μ m in height, and having stout stalks (Fig. 33-35). Spores mostly irregular, with flattened or concave sides, and surrounded by a thin spore wall, 3.8-9.2 μ m in diameter. Myxamoeba aggregating into the center not by streaming but by movement singly or in small groups. The resulting shield-shaped pseudoplasmodium giving rise to one whitish, simple or compound sorocarp. Spores



Figs. 23-29. Charateristics of P-pallidum, Fig. 23 Branched fruiting body. $\times 40$, Fig. 24 Elongation of stalk, $\times 40$, Fig. 25 Initiation of branching. $\times 100$, Fig. 26 Base. $\times 200$, Fig. 27 Spores. $\times 1000$, Fig. 28 Aggregation streaming. $\times 100$, Fig. 29 Sori and pseudoplasmodium. $\times 40$.



Figs. 30-32. Copromyxella sp., Fig. 30 Irregular shaped fruiting body. $\times 100$, Fig. 31 Fruiting body. $\times 200$, Fig. 32 Fruiting bodies are branched profusely. $\times 200$, Fig. 33-34 G. vulgaris, Fig. 33 Fruiting bodies are milk-white in color. $\times 35$, Fig. 34 Lateral view of fruiting bodies. $\times 20$, Fig. 35 Head-shaped fruiting body. $\times 100$.

Table 2. Distribution of cellular slime molds in deciduous forests

					Symbols	of sites	3					
Species	CR DY UL KR SL DJ NH YM CM							СМ	Avg.			
						DJ		1 1/1	CIVI	MN	Freq.	F(%)a
				Sa	mple Fre	quency	(%)					
D. minutum	30	10	_		80	100	100	60	20	40	44	80
D. mucoroides	80	40	80	20	60	_	40	20	_	80	42	80
P. violaceum	20	_	-	60	80	_	20	60	40	-	28	60
P. pallidum	60	40	_	20	60	_	_	40	80		30	60
G. vulgaris	20	_	10	_		10	10		40	40	13	60
Dictyostelium sp.	_	_	_	-		20	40	20	_		8	30
D. lacteum	_	20	_	30		_	_	_	_	5	20	
D. fasciculutum	_	-	_	_		_	10	40	~	_	5	20
D. polycephalum	-	_	_	_	10	_		_	_		1	10
Copromyxella sp.	_	_	_			_	_	_		80	8	10
No. of species/ Site	5	4	2	4	5	3	6	6	4	4	Avg. = 4.3	

a. Site frequency

CR: Mt. Chiri, DY: Mt. Dugyou, UL: Ulleung Island, KR: Mt. Keryong, SL: Mt. Songli, DJ: Dugjuk Island, NH: Mt. Namhan, YN: Mt. Yongmoon, CM: Mt. Chunma, MN: Mt. Mani

distinguished by their spherical irregularities.

Distribution. D. minutum and D. mucoroides were the most commonly found in ten deciduous forests soils (Table 2). The two species represented the same 80% site frequency. P. violaceum, P. pallidum and G. vugaris occurred commonly in six sampling sites, showed 60% site frequency. These species were second in importance. D. lacteum and D. fasciculutum occurred in two sites, showed 20% frequency each. G. vulgaris showed low sample frequency (average 13%), but it was comparatively high in site frequency (60%). D. polycephalum and Copromyxella sp. were found only in one site-Mt. Songli and Mt. Mani respectively.

Copromyxella sp., which could not be identified, represented 80% sample frequency only in one site-Mt. Mani. Dictyostelium sp., the other species not identified, represented 30% site frequency. D. minutum, D. mucoroides, P. violaceum, P. pallidum and G. vulgaris were widely distributed in deciduous forests. D. fasciculutum, and Dictyostelium sp. and Copromyxella sp. were found mainly in the upper sampling areas. They were not found in Mt. Chiri, Mt. Dugyou, Mt. Songli, Mt. Keryong and Ulleung Island. In the two islands, cellular slime molds were rarely found, except D. minutum. Only D. vulgaris occurred in both islands infrequently (10% sample frequency). Number of isolates per sampling sites ranged from 2 to 6, averaging 4.3. Diversity of species was comparatively high in Mt. Yongmoon, but there was not any great difference among the sampling sites.

DISCUSSION

Cellular slime molds were found to occur frequently and abundantly in forest soils, their primary habitat (Cavender and Raper, 1965b,c). However, there are no reports on the acrasian populations and their distribution in forest type in Korea. Therefore little was known concerning their presence in habitat at the beginning of this study.

Three families, four genera, and ten species were isolated from soils of ten sampling sites including 8 mountains and 2 islands of Korea. The number of isolates is almost similar to that of Switzerland (11 species; Traub *et al.*, 1981), eastern Canada (9 species; Cavender, 1972), Southeast Asia (average 9.8 species; Cavender, 1976a), Alaska (9 species; Cavender, 1977) and East Africa (9 species; Cavender, 1969b).

D. minutum and D. mucoroides occurred pre-

dominantly in deciduous forests soils. The occurrence frequency of them was judged to be higher than that of any other species (Table 2). D. mucroroides forms a complex of perhaps four species (Cavender, 1972): D. mucoroides, D. giganteum. D. aureo-stips and D. sphaerocephalum. These species, originally described Brefeld (1869), were reported to widespread occur in many countries. It was very difficult to identify these D. mucoroides complex because of their resemblances of spherical appearance. D. minutum rarely occurred in southern California (Benson and Mahoney, 1977) and subtropical and tropical America (Cavender and Raper, 1968), and it was not even found in Southeast Asia (Cavender, 1976b). However, it was reported that D. minutum was found abundantly in tundra and forest soils of Alaska (Cavender, 1977), eastern North America (Cavender and Raper, 1965c) and eastern Canada (Cavender, 1972). This indicates that D. minutum prefers to prehaps boreal forest soils, and grows well in cooler regions.

D. polycephalum is known as a species which is most ecologically distinct (Cavender and Raper, 1965c), and an "indicator" of a particular forest type, lowland hardwood forests. According to Cavender (1972), it occurred most frequently in temperature areas, but it was rerely found in this study. Similarly, D. lacteum, D. fasciculutum and Copromyxella sp. rarely occurred in Korean deciduous forests. D. fasciculutum, which sorocarp resembles D. mucoroides when solitarily appeared, was not found in any countries of Asia, but it was found in Mt. Namhan and Mt. Yongmoon of Korea. It was first reported by Traub et al. (1981a) that it occurred abundantly in Switzerland. It is not clear that Corpromyxella sp., whose sorocarp is delicate and irregular, belongs to the genus Copromyxella, but it most resembles C. coralloides Raper and Kurzynski in superficial appearance. It is suggested that a more detailed study of its characteristics be needed. Dictyostelium sp., probably the other new specis, is conspicuously different from other species in forms and lengths of spores or bases, and we will report it later after further observations.

P. violoceum and P. pallidum, which easily identified because of distinct characteristics of their sorocarps, were commonly found in these study areas. Especially, two species occurred nearly in the same sites at the same time. It is probably because they have some identity in preference of locality. The last clones, G. vulgaris are conspicuously different from well-known

appearance of cellular slime molds and it is easily found in six forests soils, but the other species belonging to the genus *Guttullinopsis*, *D. nivea* (Raper and Worley, 1977) was absent in any sites investigated in this study. *D. discoideum* Raper widely known as a representative species of Acrasieae was notably absent in these study areas.

The numbers of cellular slime molds isolated per sampling site ranged from 2 to 6, averaging 4.3. Notably, only two speciess were found in Ulleng Island, differently from expectation. It was probably because during collected samples were transported to laboratory they were dried, spores and myxamoeba were diminished. Diversity of isolates was comparatively high in Mt. Yongmoon, but there was no great difference in numbers of isolated organisms among the sampling sites.

적 요

전국의 10개 주요 낙엽수림에서 채집한 시료로부터 세포성 점균은 성 점균의 출현과 분포를 조사하였다. 세포성 점균은 clonal isolation technique 방법에 의하여 토양으로부터 분리되었으며, E. coli 와 함께 건초 한천 배지에서 배양되었다. 그 결과 3과 4속 10종이 발견되었으며 다음과 같이 기록한다: Dictyostelium minutum Raper, Dictyoslelium mucoroides Brefeld, Dictyostelium polycephalum Raper, Dictyostelium lacteum Van Tiegham, Dictyostelium faciculutum Traub, Hohl and Cavender, Dictyostelium sp., Polysphondylium violaceum Brefeld, Polysphondylium pallidum Olive, Copromyxella sp. 그리고 Guttulinopsis vulgaris Olive. 그 중 Dictyostelium sp.와 Copromyxella sp.는 현재 확인되지 않은 종이다.

조사된 낙엽림에서 가장 흔하게 출현하는 우점종은 D. minutum 과 D. mucoroides 로 나타났다. Dictyostelium sp., D. fasciculutum 그리고 Copromyxella sp.는 용문산과 남한산 등 비교적 북위도상의 낙엽림에서만 발견되었으나 D. lacteum 과 D. polycephalum은 중부 이남에서만 나타나고 있었다. 이같은 결과는 이들 종의 분포가 기후에 영향을 받고 있음을 시사하고 있다. 각 조사지역에서 2-6종이 발견되었으며, 채집지역별 평균 출현종 수는 4.3으로나타났다.

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