식물학회지 제35권 제3호
Korean J. Bot. 35(3) : 197~203

A New Species of Cellular Slime Molds from Korea, *Dictyostelium flavidum* sp. nov.

Hong, Jeong-Soo and Nam-Kee Chang
(Department of Biology Education, Seoul National University, Seoul, Korea)

韓國產 細胞性 粘菌의 1 新種 *Dictyostelium flavidum* sp. nov.

洪 廷 守·張 楠 基
(서울대학교 사범대학 생물교육과)

ABSTRACT

During the study of the distribution of cellular slime molds in Halla mountain of Korea, a new yellow-pigmented *Dictyostelium* was isloated. This exibited several distinctive features which differed from the published species, and was designated as a new species, *Dictyostelium flavidum* sp. n. Hong et Chang (Type strain HL-1). It was cultivated at 20-22°C on weak nutrient agar media, 0.1 L-P in association with *Escherichia coli*. Sorocarps were 4-10 mm or more in length, conspicuously yellow throughout or with sori, typically solitary, unbranched or sparsely and irregularly branched. Sorophores were strongly tapered from bases to tips. Bases were typically well-formed disks in form or conically expanded. Sori were yellow to yellowish in color, and the pigmentation intensified with age. Spores were long and thin elliptical, mostly 4.8-9.6×1.9-3.8 (ave. 7.3×2.8) μm, L/B index about 2.4-2.8, without polar granules.

INTRODUCTION

Cellular slime molds are widely distributed in nature, but are believed to be distributed differently in relation to environmental conditions. Occurrence and distribution of these organisms have been investigated in many countries and various forest types (Cavender and Kawabe, 1989; Landolt and Stephenson, 1990; Stephenson *et al.*, 1991; Hong and Chang, 1990, 1991; Hong *et al.*, 1992a, b). From these studies, many new species were continually reported, and these species have been taxonomically reviewed (Bonner, 1967; Olive, 1975; Traub and Hohl, 1976; Raper, 1984; Hagiwara, 1989).

During the study of the distribution of cellular slime molds in Halla mountain of Korea, many undescribed species, including a new yellow-pigmented *Dictyostelium*, were isloated. This new species exibited several distinctive features which differed from the published species and here was described as a new species, *Dictyostelium flavidum* Hong et Chang sp. n..

The golden-yellow pigmented *Dictyostelia* have been isolated from soils of tropical evergreen rain forest and semi-deciduous forests of South America (Cavender, 1970). Later, additional yellow-pigmented *Dictyostelia* were isolated from chaparral in southern California (Benson and Mahoney, 1977) and deciduous forest in Switzerland (Traub *et al.*, 1981). Meanwhile Cavender *et al.* (1981) described two varieties and a new species. *Dictyostelium flavidum* sp. n. Hong et Chang is clearly different from these yellow-pigmented species not only in morphological features but also in developmental and growth habits.

MATERIALS AND METHODS

Cellular slime molds isolated were grown on weak nutrient media, 0.1% lactose-peptone agar (0.1 L-P), consis-

ting of 1.0 g lactose, 1.0 g peptone, 1.5 g KH_2PO_4, 0.96 g $Na_2HPO_4 \cdot 12H_2O$ and 20 g agar/*l* distilled water, adjusted to pH 6. *Escherichia coli* were used as the most desirable food organism (Cavender, 1976) and were already cultured on the liquid medium for overnight with a rotary shaker at 37°C. These were centrifuged at 3000 rpm for 5 min, and the high suspension of fresh bacteria were prepared and stocked at 4°C. Spores were usually inoculated at the center of cross streaks made with a suspension of bacteria and were incubated at 20-22°C in diffuse light and darkness. For the study of aggregation pattern, a suspension of spores and bacteria was spread as a broad band across the agar surface. Both growth and development were observed. Photographs were taken with a photomicroscope.

RESULTS

Description: *Dictyostelium flavidum* sp. n. Hong et Chang. Cultum ad 20-22°C in diluto "lactose-peptone" agaro cum *Escherichia coli*; sorocarpi insigniter flavicolore ominio, typice solitarii vel gregarii, primo erecti vel semierecti, grandi, non ramosi vel exigue et inaequaliter ramosi; aggregationes et sorogena flavicolore; pseudoplasmodia typice 195-439 μm in diam; sorophora flavo usque hebeti et luteolo colore, typice longa et fortia, vel sinuosi, 4-10 mm in longitudinem et 1-4 inaequaliter locatos laterales ramos gerentia, aliquando ad 3 cm in longitudinem, duobus vel pluribus pedunculis saepe adhaerentibus, pedunculis 26-95 μm in basi usque 1.2-4.0 μm prope apicem valde attenuatis, incohatis usque bene formatis basalibus discis aliquando stabiliti; Basi typice bene formatis discis vel conus; rami plerumque 0.4-6 mm in longitudinem; sori flavo usque hebeti et luteolo colore, grandi, globosi usque paene citriformes, teminales sori 250-500 μm, laterales sori 30-222 μm; sporae longae et tenues ellipticae, plerumque 4.8-9.6×1.9-3.8 μm (mediae dimensiones 7.3×2.8 μm), sine granulis polaribus.

Holotypus — Stirpes typicae HL-1 e solo Hallasan, Cheju-do, Korea.

Cultivated at 20-22°C on weak nutrient agar media, 0.1 L-P in association with *Escherichia coli*. Sorocarps conspicuously yellow throughout or with sori pale yellow in some isolates, typically solitary but occasionally clustered, mostly erect or semierect but sometimes prostrate, robust, unbranched or sparsely and irregularly branched (Figs. 14-16). Aggregations and sorogens in yellow shades. Aggregates small mound in form (Fig. 3), and larger with faintly diffuse streams with time (Figs. 4 and 5). Pseudoplasmodia compact round in form (Fig. 6), mostly 195-435 μm in diam. Sorogens without stalk-free migration (Figs. 11 and 12). Sorophores yellow to yellowish, or colorless, typically large and stout or slightly sinuous, mostly 4-10 mm, sometimes up to 3 cm or more in length, with two or more stalks often adherent, strongly tapered from bases to tips (Figs. 16 and 17) measuring 26-95 μm at the base and mostly 1.2-4.0 μm near the apex, often anchored in rudimentary to well-formed basal disks (Fig. 18). Bases typically well-formed disks (Figs. 19 and 20) or expanded conical but somtimes round surrounded by conical slime mass. Tips mostly obtuse or capitate (Figs. 21 and 22), but sometimes acuminate. Sori yellow to yellowish in color, large, the pigmentation intensifying with age, globose to citriform, primary sori 250-500 μm in diam. and lateral sori 30-222 μm in diam. Spores hyaline, long and thin elliptical (Figs. 1 and 2), mostly 4.8-9.6×1.9-3.8 (ave. 7.3×2.8) μm, L/B index about 2.4-2.8, without polar granules. Macrocysts and microcysts not observed.

Holotype — Type strain HL-1 was originally isolated, in 1989 by J. S. Hong and N. K. Chang, from the soils collected in cool temperate deciduous forest of Halla mountain, Cheju island, Korea.

Korean name: 노랑장대팡이

Observations. Spores of *D. flavidum* sp. n. (Figs. 1 and 2) are very characteristic in comparison with other yellow-pigmented species, namely *D. areum* var *aureum*, *D. aureum* var. *luteolum*, and *D. mexicanum*. Myxamoebae aggregate to form small mound without inflowing streams (Fig. 3). The mounds grow gradually larger due to the centralizing of myxamoebae, and the faintly diffuse streams often appear (Figs. 4 and 5). The pseudoplasmodia are typically compact round in pattern with indefinite streams (Fig. 6). An aggregate typically forms a single sorogen (Fig. 6) but occasionally produces a cluster (Fig. 7). Pseudoplasmodia are faintly yellowish in color, mostly 195-435 or more in diam., and often show a 'mexican hat' as in *D. discoideum*. These arise from the center (Fig. 8), and produce a conical mound (Fig. 9) of a club-like form (Fig. 10). When the sorogens rise, a few streams are sometimes distinctive (Fig. 10). Sorogens rise directly under normal conditions (Fig. 11) but sometimes prostrate without a stalk-free migration. Developing sorogens occasinally produce adherent stalks (Fig. 12). Some slime substances are often attached around the base of developing sorophores, showing a 'spider's web'.

As the sorogens develope, the centralizing of myxamoebae continues. A mass of myxamoebae, which aggre-

gate late, climbs an unmatured sorocarp, and a thick secondary structure of slime mass is formed around the base (Fig. 13) and develops into a single branch (Fig. 14). Then another late mass climbs the branch through the basal part of the sorophore and develops into a second branch near the base of the first branch (Fig. 15). However, all branches are not formed in this pattern. Sometimes a small mass of myxamoebae is separated from the rising or prostrating sorogen, and soon comes to develop into a single branch or irregularly into two or more branches. In addition, occasionally two or more contiguous sorogens interwind to ascend and form coremium-like sorophores, subsequently becoming separated irregularly. Bases are very thick and stout and often surrounded by aprons of slime (Fig. 17). Small sorocarps are much more thick and stout, and may show a gradual reduction in width from bases to tips. However, the diameter decrease markedly in typically large sorocarps. Sorophores are often slightly curved near the bases (Fig. 18) and sometimes fall down with a straight form on the agar plate. Therefore the bottom of basal disks are often oriented toward the side. This habit makes the identification of basal disks on the agar plate easy (Fig. 19). are typically well-formed discs (Fig. 20) or cones, and the slime debris is often tightly attached, mostly 26-95 μm in diameter. Occasionally bases are round in form surrounded by conically expanded slime mass. Tips are very thin and delicate in most typical sorocarps (Figs. 21 and 22), but sometimes vary in form. The pigmentation of sori intensifies with age, particularly in large sori.

DISCUSSION

Dictyostelium flavidum sp. n. (HL-1) is characterized by its golden-yellow sori, large and stout sorocarps, unbranched or sparsely and irregularly branched, and strongly tapered sorophores, well-formed basal disks, and narrowly elliptical spores without polar granules. Therefore it can be easily recognized and distinguished from the other species on the basis of its morphology. This species was, in 1989, originally isolated from the soils of Mt. Halla and repeatedly found in warm temperate forests of Cheju island (Hong *et al.*, 1992a, b). Later, it was also found in the other deciduous forest soils of Korea (Hong *et al*, unpublished data). It may distribute widely in Korean forest soils. The genus *Dictyostelium*, established by Brefeld in 1869, is characterized by fructifications or sorocarps that are typically erect or semeeroct and unbranched or sparsely and irregularly branched. The stalks or sorophores consist of strongly vacuolate parenchyma-like cells compacted within a continuous cellulose sheath, and they bear globose to citriform spore masses at their apices or sori (Raper and Fennell, 1967). Hence, this new species belongs to the *Dictyostelium*.

Cavender *et al.*(1981) described three golden-yellow dictyostelids. These are two varieties and a new species, that is *D. aureum* var. *aureum*, *D. aureum* var. *luteolum*, and *D. mexicanum*. *D. flavidum* sp. n. apparently resembles the two *D. aureum* varieties in yellow pigmentation, large sorocarps, and PG negative spores. *D. flavidum* sp. n. is, however, clearly different from the two in spore forms, aggregation patterns, basal forms, and sorophores. *D. aureum* var. *aureum* has relatively small elliptical or slightly reniform spores, mostly 5.0-6.4×3.0-3.5 μm. *D. aureum* var. *luteolum* has broadly elliptical to oval spores, commonly 6.0-7.0×3.5-4.5 μm. These two varieties show commonly dendritic streams of myxamoeba during the stage of aggregation, mostly club-like bases. Sorophores of the two are comparatively thin, little tapered, much more flexuous and often tangled. *D. flavidum* has clearly longer and thiner spores (Figs. 1 and 2), aggregates with indefinite or faintly diffuse streams (Figs. 3-5), and typically well-formed basal disks (Figs. 19-20). Sorophores of *D. flavidum* are thick, strongly tapered, and robust (Fig. 16). *D. mexicanum* has PG positive spores and much smaller sorocarps.

D. flavidum resembles *D. firmibasis* (Hagiwara, 1989) in its large sorocarps and narrowly elliptical spores. However, *D. firmibasis* lacks distinctive yellow-pigmentation, well-formed basal disks and irregular branches. Early sorogens, spore forms, and well-formed basal disks of *D. flavidum* are very similar to those of *D. discoideum*, particularly in its basal disks. *D. flavidum* with unbranched sorophore shows a very large form of *D. discoideum*. However, *D. flavidum* does not show stalk-free migration, which is characteristic of *D. discoideum*. In addition, *D. discoideum* is much small, and unbranched.

적 요

한라산의 삼림토양에서 하나의 새로운 세포성 점균이 발견되었다. 이 종의 1차적 특징은 황색의 포자낭과 큰 자실체이므로 학명을 *Dictyostelium flavidum* sp. n. Hong et Chang으로, 그리고 한국명을 '노랑장대팡이'로 명명하였다. 이 종은 20-22°C의 0.1 L-P(pH 6)에서 *Escherichia coli*와 함께 이원 배양되었다. *D. flavidum* sp. n.의 자실체는 대부분 4-10 mm 혹은 그 이상으로서 다른 종에 비해 매우 크며, 튼튼하고, 곧거나 비스듬히 서며, 전형적으로 단생

하나 가끔 군생하고, 가지가 없거나 불규칙하다. 초기 집합체는 작은 덩어리 모양이며, 집합줄기는 희미한 방사형을 나타내나 종종 보이지 않는다. 위원형체는 연황색을 나타내고, 흔히 탄탄한 구형 혹은 타원형이며, 자루없는 이동을 하지 않으며, 이 것이 위로 성장함에 따라 집합줄기가 종종 뚜렷해 진다. 자루는 연황색 혹은 무색이며, 기부에서 정단으로 갈수록 매우 가늘어진다. 또한 2-3개의 자루가 종종 붙은 채로 형성되기도 한다. 기부는 전형적으로 잘발달된 원반형 혹은 원뿔형이며, 종종 원뿔형의 점액질로 둘러싸인 구형이 나타나기도 한다. 포자낭은 전형적으로 황색 혹은 연황색이며, 시간이 지날수록 색깔이 더욱 뚜렷해 지는 경향이 있고, 주 자루의 포자낭 직경은 흔히 250-500 μm로서 매우 크다. 포자는 PG가 없고, 가늘고 긴 타원형이며, 크기는 4.8-9.6×1.9-3.8 (ave. 7.3×2.8) μm로서 L/B 계수는 2.4-2.8 이다. *D. flavidum* sp. n.은 황색의 포자낭, 매우 크고 튼튼한 자실체, 원반형의 기부, 그리고 PG가 없고 가늘고 긴 형의 포자에 의해 특징된다. 이 종은 한라산의 냉온대 낙엽수림에서 처음 발견되었으나 제주도의 난온대 상록수림과 내륙의 낙엽수림에서도 반복적으로 분리되어 우리나라 삼림 토양의 특징종으로 여겨진다. 이 종은 종설명과 함께 이미 발표된 유사한 종들과 비교되었다.

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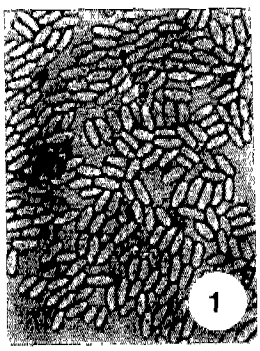
(Received May 20, 1991)

Explanation of Figures

Fig. 1. Narrowly elliptical spores, ×400.
Fig. 2. Spores without polar granules, ×1000.
Fig. 3. Early stage of aggregation showing a small mound without inflowing streams, ×40.
Fig. 4. Centralizing of myxamoebae showing faintly diffuse streams.×40.
Fig. 5. Late stage of aggregation, ×40.
Fig. 6. Pseudoplasmodia showing often compact forms with indefinite streams, ×40.
Fig. 7. Clustered sorogens, ×40.
Figs. 8-10. Forms of a rising sorogen with time, ×40. A few of streams are sometimes distinctive(arrow).
Fig. 11. A developing sorogen rising directly, ×30.
Fig. 12. Sorogen producing adherent stalks, ×40.
Fig. 13. A slime mass climbing an immature sorocarp, ×40.
Fig. 14. Banching habit, ×40.
Fig. 15. Detail of branching habit, ×40. Note primary and secondary branches developed by a slime mass which climbs an sorocarp.
Fig. 16. Sorocarps unbranched or sparsely and irregularly branched, ×20. Note the strongly tapered sorophores.
Fig. 17. A thick and stout base surrounded by aprons of slime, ×100.
Fig. 18. Sorophores slightly curved near the base, ×40. A basal disk is easily recognized on the agar plate.
Fig. 19. Details of a basal disk on the agar plate, ×100.
Fig. 20. Typically well-formed basal disks, ×100.
Fig. 21. Obtuse tip, ×100.
Fig. 22. A small capitate tip, ×100.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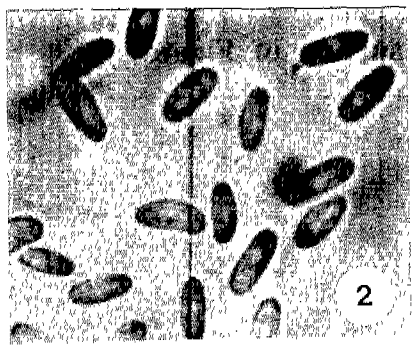
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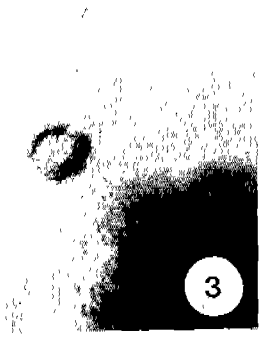
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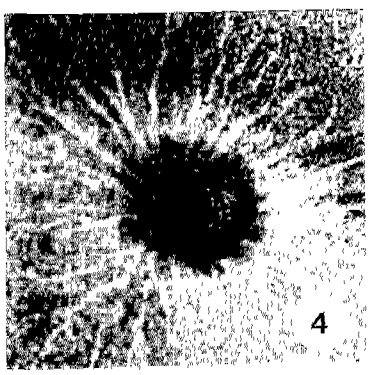
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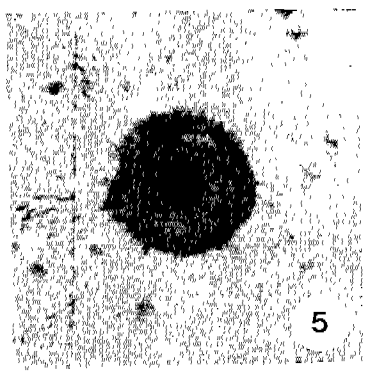
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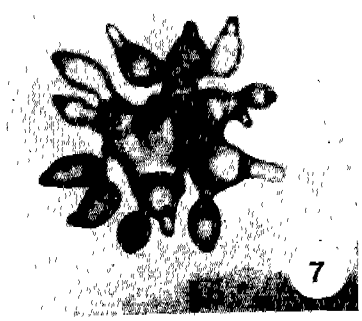
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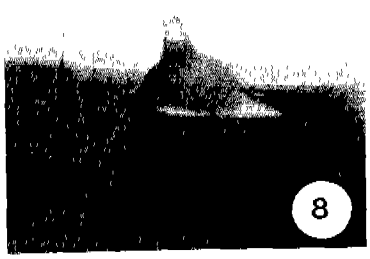
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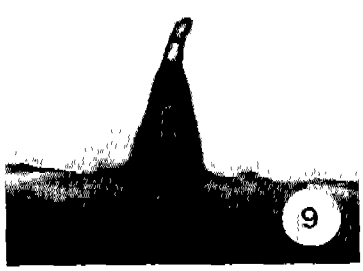
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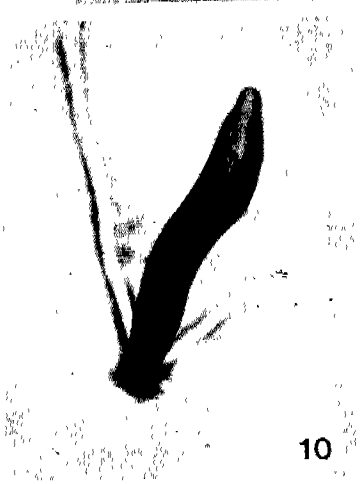
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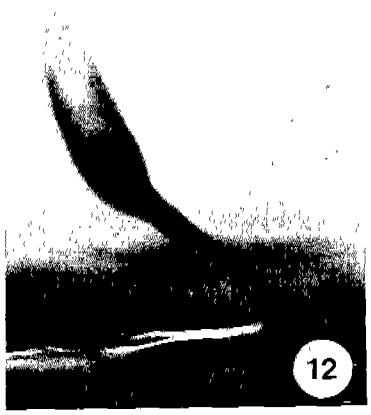
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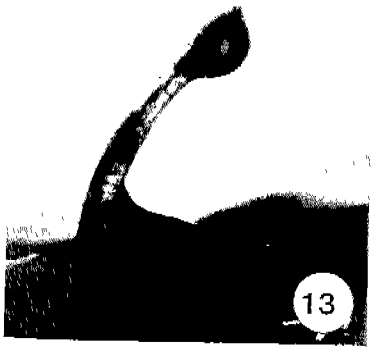
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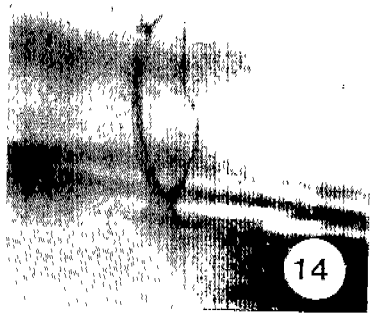
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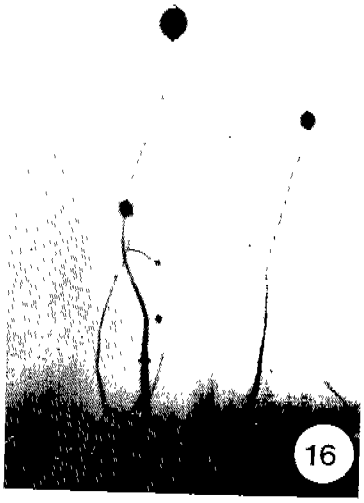
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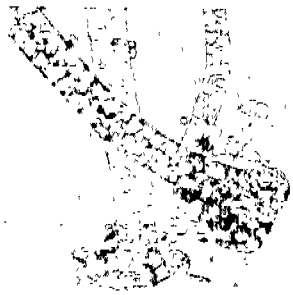
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