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Laboratory Cultures of Myxomycetes: Fruit Body Formation in Five Species of the Genus *Didymium*

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Abstract Laboratory cultures of myxomycetes of the genus *Didymium* were investigated. Formation of fruit bodies and spore-to-spore cultivations were completed for five species, *Didymium bahiense* var. *bahiense*, *D. minus*, *D. marineri*, *D. iridis*, and *D. flexuosum*.

Key words: Myxomycetes, *Didymium bahiense* var. *bahiense*, *D. minus*, *D. marineri*, *D. iridis*, *D. flexuosum*, fruit body, plate culture.

Laboratory cultures of myxomycetes were investigated from the viewpoint of search for bioactive secondary metabolites. We recently isolated makaluvamine A, a highly cytotoxic topoisomerase II inhibitor, from the plasmodial culture of *Didymium bahiense* var. *bahiense* Gottsb. (Ishibashi *et al.*, 2001). On the other hand, we further examined the formation of spores or fruit bodies from plasmodia of several myxomycete species in plate cultures in order to achieve spore-to-spore cultivations of as many myxomycete strains as possible. As a result, we recently found formation of fruit bodies of five species, *Didymium bahiense* var. *bahiense*, *D. minus* (Lister) Morgan, *D. marineri* Moreno, Heykoop & Illana, *D. iridis* (Ditmar) Fr., and *D. flexuosum* Yamashiro in plate agar cultures containing oatmeals.

Materials and Methods

1. Myxomycetes organisms

The fruit bodies of the myxomycete *Didymium bahiense* var. *bahiense* (Order Physarales; Family Didymiaceae, Fig. 1a) and *D. minus* (Fig. 2a) were collected at Ina, Nagano Prefecture, Japan, in August, 1999. *D. marineri* (Fig. 3a) and *D. iridis* (Fig. 4a) were collected at Rakansan, Yamaguchi Prefecture, Japan, in July 2000, and *D. flexuosum* (Fig. 5a) was collected at Hiyoshi, Yokohama, Kanagawa Prefecture, Japan, in June, 2000.

2. Culture conditions

The spores from the fruit bodies were applied on agar plates containing the following media A or B (media A: KH_2PO_4 1.45%, $Na_2HPO_4 \cdot 12H_2O$ 2.4%, agar 1.0%; media B: lactose 0.1%, peptone 0.1%, KH_2PO_4 0.205%, $Na_2HPO_4 \cdot 12H_2O$ 0.083%, agar 1.5%) with a suspension of *Escherichia coli* (0.1 mL in Nutrient media or Heart Infusion media, DIFCO). After static incubation at 22 or 25℃ in the dark condition for 4-5 days, myxamoebic plaque appeared, and the plaque was transferred several times to new agar plates containing the media B until the plasmodial formation was observed (Figs. 1b-5b). The plasmodia were then cultured in agar plates (the media B) with oatmeal (*ca.* 0.2 g/plate, autoclaved prior to use) for 1-2 weeks at 25℃ in the dark condition.

Results

The plasmodial cultures of these five strains successfully developed into spores in the presence or absence of light, implying that spore-to-spore cultivation, i.e., rotation

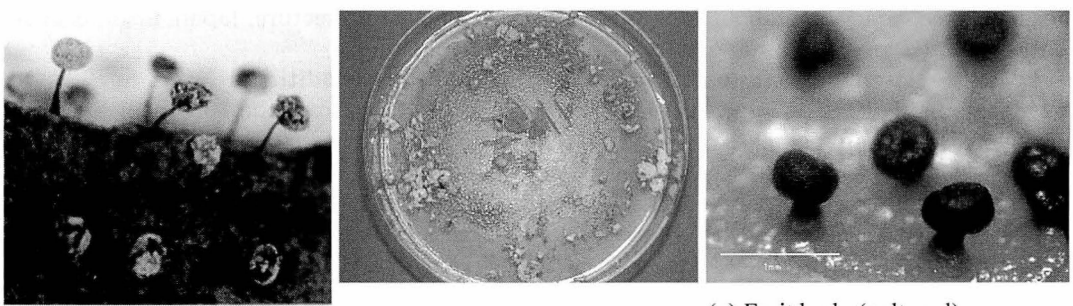
of one life cycle, was realized on agar plates. In addition, for these five strains, formation of fruit bodies were observed on the oatmeal agar plates (Figs. 1c–5c). The fruit body formation from cultured plasmodia of each species was observed as follows.

***Didymium bahiense* var. *bahiense* Gottsb.** (Fig. 1): The cultured plasmodia were brown and grown under dark condition at 25℃ in agar plates with media B in the presence of oatmeal. After the plasmodia have spread over the 9-cm plate for 1 week, the plate was placed at room temperature under natural light. In 2–3 days the brown color of the plasmodia disappeared and the formation of fruit bodies was observed.

***Didymium minus* (Lister) Morgan** (Fig. 2): The cultured plasmodia were white and grown under dark condition at 25℃ in agar plates with media B in the presence of oatmeal. After the plasmodia have spread over the 9-cm plate for 1–2 weeks, the plate was still placed at 25℃ under dark condition, and after 1 week, the formation of fruit bodies was observed.

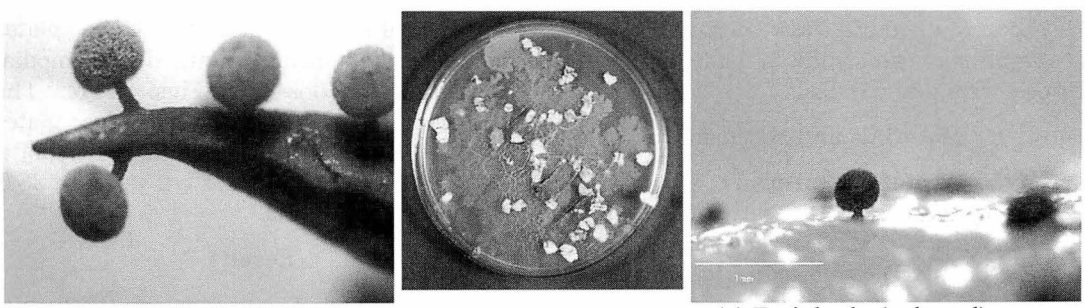
***Didymium marineri* Moreno, Heykoop & Illana** (Fig. 3): The cultured plasmodia were white and grown under dark condition at 25℃ in agar plates with media B in the presence of oatmeal. After the plasmodia have spread over the 9-cm plate for 1 week, the plate was placed at 22℃ in a cycle of 16-h light and 8-h dark. In 1 day the color of plasmodia changed into yellow, and in another 3–4 days, the formation of fruit bodies was observed.

***Didymium iridis* (Ditmar) Fr.** (Fig. 4): The cultured plasmodia were lemon-colored and grown under dark condition at 25℃ in agar plates with media B in the presence of oatmeal. After the plasmodia have spread over the 9-cm plate for 2 weeks, the plate was placed at 22℃ in a cycle of 16-h light and 8-h dark. In 1 week, the formation of fruit bodies



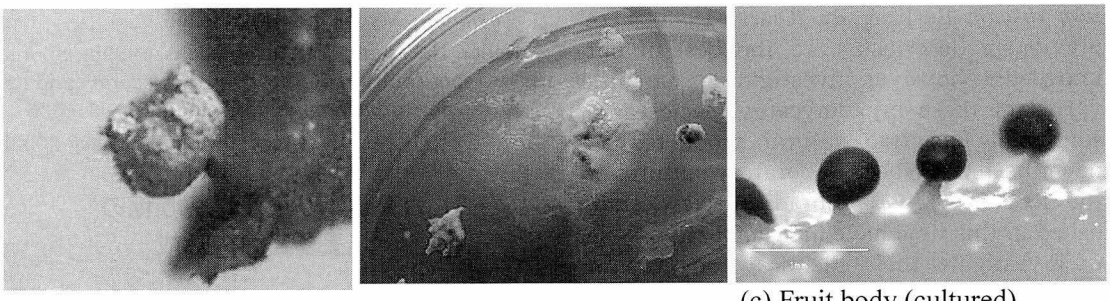
(a) Fruit body (wild). (b) Plasmodium (cultured). (c) Fruit body (cultured). (Bar represents 1 mm.)

Fig. 1. *Didymium bahiense* var. *bahiense.*



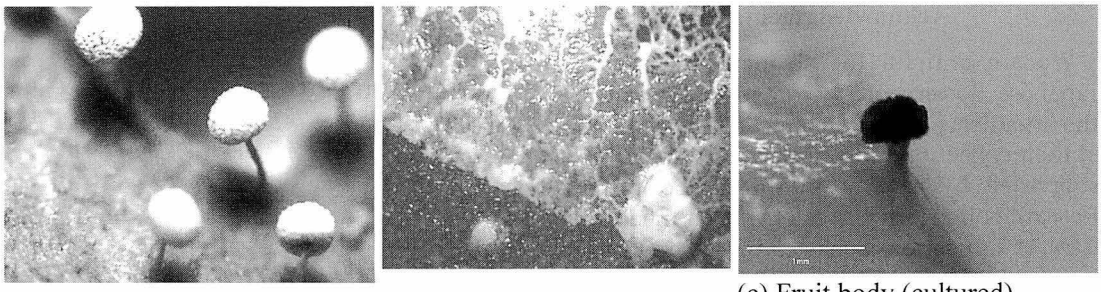
(a) Fruit body (wild). (b) Plasmodium (cultured). (c) Fruit body (cultured). (Bar represents 1 mm.)

Fig. 2. *Didymium minus.*



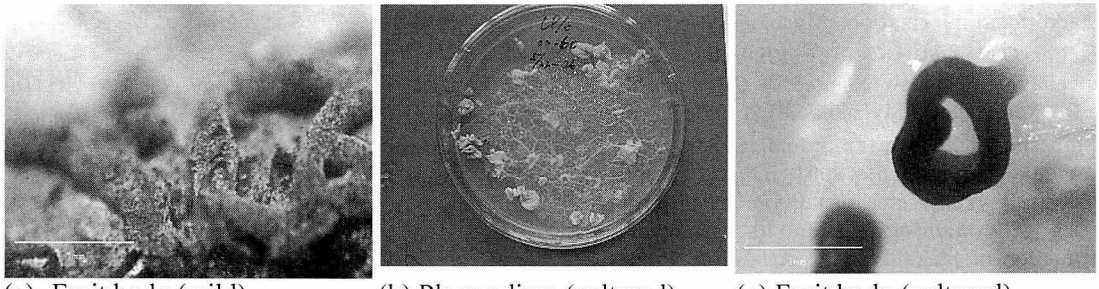
(a) Fruit body (wild). (b) Plasmodium (cultured). (c) Fruit body (cultured). (Bar represents 1 mm.)

Fig. 3. *Didymium marineri.*



(a) Fruit body (wild). (b) Plasmodium (cultured). (c) Fruit body (cultured). (Bar represents 1 mm.)

Fig. 4. *Didymium iridis.*



(a) Fruit body (wild). (Bar represents 1 mm.) (b) Plasmodium (cultured). (c) Fruit body (cultured). (Bar represents 1 mm.)

Fig. 5. *Didymium flexuosum.*

was observed.

***Didymium flexuosum* Yamashiro** (Fig. 5): The cultured plasmodia were white and grown under dark condition at 25℃ in agar plates with media B in the presence of oatmeal. After the plasmodia have spread over the 9-cm plate for 5 days, the plate was placed at 22℃ in a cycle of 16-h light and 8-h dark. In 1 day the color of plasmodia changed into yellow, and in another 2 days, the formation of fruit bodies was observed.

Discussion

Reproductive behavior of *Didymium iridis* was previously reported (Collins and Tang, 1989). For other species, spore-to-spore cultivation or fruit body formation of *D. annulisporum* Keller & Schoknecht (Keller and Schoknecht, 1989a), *Badhamia spinispora* (Eliasson & Lundqvist) Keller & Schoknecht (Keller and Schoknecht, 1989b), and *Physa-*

rum roseum Berk. & Br. (Clark, 1995) were previously described. To the best of our knowledge, however, investigations on cultivation of these myxomycetes species as a source of bioactive natural products had never been described. Laboratory cultures of the five *Didymium* Schrad. species established in this time have first make it possible to investigate their chemical constituents. Studies on large-scale culture of these *Didymium* Schrad. species as well as analyses of their chemical constituents are currently under investigation in our laboratories.

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カタホコリ属変形菌5種の培養子実体の形成

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天然資源からの有用生物活性物質探索の一環として，変形菌（真性粘菌）の培養菌株コレクションの作製をめざして変形菌の実験室内での培養を試みた．まず，寒天平板培地上で大腸菌の存在下，野外調査・採取により得た変形菌の子実体に含まれる胞子の発芽実験を行った．次に溶菌斑として胞子の発芽（粘菌アメーバの形成）が観察された菌株について，オートミール寒天培地への植え継ぎを繰り返し，変形体の形成ならびに子実体の形成を試みる実験を行った．その結果，今回，国内で採取した5種のカタホコリ属（*Didymium* 属）に属する変形菌（*Didymium bahiense* var. *bahiense, D. minus, D. marineri, D. iridis,* および *D. flexuosum*）について，寒天平板培地上での変形体の培養ならびに子実体の形成に成功した．これにより，これら5種のカタホコリ属変形菌について，平板培地上での大量培養ならびにそれらの化学成分に関する研究を行うことが可能となった．