Short Notes

OCCURRENCE OF CELLULAR SLIME MOLDS (DICTYOSTELIDS) IN SUBIC BAY NATURAL FOREST RESERVE, ZAMBALES, PHILIPPINES

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ABSTRACT

Cellular slime molds or dictyostelids are unicellular, amoeboid organisms that feed on bacteria. They are commonly found in forest soils where they play an important role in maintaining balance among soil microbial flora. However, in the Philippines, in spite of their important ecological roles, very few studies have looked at these organisms. Thus, this present investigation was designed to look at the occurrence of cellular slime molds in two forest trails within Subic Bay Natural Forest Reserve, Zambales, Philippines. Forest and mossy soils were collected from these areas and used for the isolation of dictyostelids. Our results showed clonal population counts of 821 to 3,150 clones/g soil. Identification of the isolated dictyostelids showed two species: *Dictyostelium laterosorum* and *Polysphondylium pallidum*. This is the first report of cellular slime molds in Subic Bay Forest Reserve, Zambales.

Keywords: clonal population count, *Dictyostelium*, diversity, forest soil, *Polysphondylium*

Cellular slime molds or dictyostelids are phagotrophic bacterivores commonly isolated from forest soils found in different climate types around the world (Swanson *et al*., 1999). These organisms are abundant in the humus layer of forest soils that are rich in bacteria on which they feed (Cavender & Raper, 1965b). They are thought to play an important role in maintaining balance among microbial communities in soil by controlling and modifying soil bacterial populations (Fenchel, 1987). At present, there are about 100 known species of dictyostelids worldwide which are grouped under three genera: *Acytostelium*, *Dictyostelium*, and *Polysphondylium*. These genera are differentiated by their stalk morphology and branching patterns.

Although cellular slime molds seem to have a global distribution, most studies about dictyostelid diversity were conducted using soils collected in Europe and the American continent (Swanson *et al.*, 1999). This means that little is known about the diversity of these organisms in other regions, e.g., in Southeast Asia. In the Philippines for instance, to the best of our knowledge, the only studies about dictyostelids have been those of Dogma & Blancaver (1965), Dogma (1975) and Cavender (1976). A total of ten species of dictyostelids are thus credited to the country: *Dictyostelium mucoroides, D. purpureum, D. lacteum* var. *papilloideum, D. mucoroides* var. *stoloniferum, D. discoideum, D. rosarium, Polysphondylium pallidum, P. violaceum, P. polycephalum,* and *Acytostelium subglobosum*. This makes assessment of dictyostelid diversity in the Philippines of great importance. Our research study looks into the cellular slime molds present in forest soil and mossy soil collected from Subic Bay Natural Forest Reserve (14° 48.336 N, 120° 19.815 E) in Zambales Province. This protected forest reserve has a tropical climate with two pronounced seasons: dry (November to May) and wet (June to October) and with annual average temperature of 26 ^{o}C. This forest habitat is characterized as lowland, dipterocarp forest. Abundant plant species include apitong (*Dipterocarpus grandiflorus*), white lauan (*Shorea contorta*), guijo (*Shorea guiso*) and palosapis (*Shorea palosapis*). No studies on dictyostelids have been so far reported from this area.

Soil samples were collected from the two forest trails within the Subic Bay Forest Reserve: the Pamulaklakin Ecotrail (190 meters above sea level, masl) and the NavMag forest area (228 masl) in August 2009 (Fig. 1). Five forest soil and five mossy soil samples (~20 g) were collected from three collection sites within each of the two forest trails, for a total of 60 soil samples. Dictyostelids were then isolated in the laboratory following the protocol of Cavender & Raper (1965a). Ten grams of each soil sample were initially diluted in 90 ml sterile distilled water. Then, five milliliters of this soil suspension were added to 7.5 ml sterile distilled water. A 0.5 ml aliquot of this soil suspension was then transferred to Hay Infusion Agar plates (HIA, boil 10 g hay/rice stalk in 1 liter water for 20 minutes) to give a final dilution of 1:50. To this, 0.4 ml of a 24-hour old *Escherichia coli* suspension was added as a food source of the dictyostelids. The bacterial and soil suspensions were then mixed together and spread over the surface of HIA plates by swirling the plates until all areas were covered. All culture plates (in triplicates per every soil sample) were incubated for 7 days under diffused light and checked for dictyostelid growth. The clonal population count was then determined by counting the number of plaques produced by dictyostelids. This number was multiplied by 50, the dilution factor of the plated soil suspension, to give the number of clones per gram of soil. The formula for this equation is as follows (Equation 1):

Equation 1:

$$\text{clonal population count} = \frac{\text{total number of clones}}{\text{1 g soil}} \times 50$$

The dictyostelids were then isolated by spore-touch technique and identified following comparison of their morphological features (i.e. the shape and size of the spores, the aggregation patterns of the streaming amoeboid cells, the shape and behavior of the forming pseudoplasmodium, and the size, color, and branching patterns of mature fruiting bodies) with published literature (Swanson *et al.,* 1999; Cavender & Vadell, 2006).

Figure 1. Map of the study site: Subic Bay Forest Reserve, Zambales, Philippines. Map source: www.wikimapia.org.

Soil pH was also determined using a pH meter (BNC pH Tester 10, Eutech Instruments). To determine the moisture content of the soil samples, 10 g of each soil sample were initially weighed and then dried at 80^{O}C for 48 hours. The dry weight was recorded and used to compute the percent moisture content of the soil (Equation 2).

Equation 2:

$$\text{\% moisture content} = \frac{\text{dry weight of soil}}{\text{original weight of soil}} \times 100$$

Our results showed that 12 soil samples from the Pamulaklakin Forest Trail and two soil samples from the NavMag Area yielded dictyostelids (Table 1). The highest clonal population count was observed from a soil sample collected in the Pamulaklakin forest trail. This soil sample had a moisture content of 14.7 % and a pH of 6.11. Shim *et al.* (1998) reported that soil pH and moisture content as well as the amount of organic material affected the species diversity of cellular slime molds as these factors influence the prey bacteria and other microorganisms present in soil. A pH range of 5.0 to 6.0 is also generally considered most favorable for dictyostelids (Stephenson *et al.,* 1999). Thus, the presence of high number of dictyostelid clones could be attributed to the physicochemical characteristics of the soil sample.

Identification of the isolated dictyostelids showed two species: *Dictyostelium laterosorum* and *Polysphondylium pallidum*. *D. laterosorum* occurred more frequently, having been isolated from 13 of 14 soil samples positive for dictyostelids (Table 1). The clonal population count ranged from 50 to 3,150 clones/g soil with an average clonal population count of 821 clones/gram soil. *P. pallidum* was isolated from only one soil sample, though the clonal population count was determined to be 2,000 clones/gram soil. The Philippines is known to be a mega hotspot of biodiversity and thus, is expected to harbor diverse species of plants and animals. However, in our research study, only two species of dictyostelids were isolated from the Pamulaklakin and the NavMag forest trails located within the Subic Bay Forest Reserve. Perhaps the bacterial population present in these forest areas may not be sufficient to sustain diverse species of dictyostelids. Soil samples with moss growth also have also been observed to have very low moisture content (Table 1). Moisture is observed to be a critical factor for the growth of dictyostelids (Shim & Chang, 1998).

Table 1. Cellular slime molds isolated from two forest trails in Subic Bay Natural Forest Reserve, Zambales.

Location	Substrate Type	Collection Code	Moisture Content (%)	pH	No. of clones (triplicate average)	Isolated Species	Clones/g of soil (triplicate average)
Pamulaklakin	Mossy Soil	SB09-MS02	5.98	6.47	14	*D. laterosorum*	700
		SB09-MS05	1.56	6.23	5	*D. laterosorum*	250
		SB09-MS07	4.56	6.50	41	*D. laterosorum*	2,050
		SB09-MS08	5.50	7.28	3	*D. laterosorum*	150
		SB09-MS09	5.08	6.83	5	*D. laterosorum*	250
	Forest Soil	SB09-FS43	17.81	6.18	29	*D. laterosorum*	1,450
		SB09-FS44	13.52	6.46	40	*P. pallidum*	2,000
		SB09-FS47	14.67	6.11	63	*D. laterosorum*	3,150
		SB09-FS49	12.94	6.41	2.33	*D. laterosorum*	117
		SB09-FS52	11.84	6.62	1	*D. laterosorum*	50
		SB09-FS53	24.61	7.10	0.67	*D. laterosorum*	34
		SB09-FS55	25.66	6.70	4.33	*D. laterosorum*	217
NavMag	Mossy Soil	SB09-MS21	7.90	6.26	1	*D. laterosorum*	50
		SB09-MS23	4.62	5.56	44	*D. laterosorum*	2,200

The different stages in the life cycle of the two isolated dictyostelids were also studied and described. *D. laterosorum* have erect sorocarps, infrequently branched, with a solitary terminal sorus and a number of lateral sori appearing along its stalk (Fig. 2). Spores are variable in shape and size but are mostly elongated, reniform, or elliptical in shape. The base of the fruiting body is digitate and crampon-like. *D. laterosorum* was first isolated by Cavender (1970) in South America and in the West Indies. Thus far, they have been isolated more frequently from soil of tropical moist forests so it was not surprising to isolate this organism in Subic Natural Forest Reserve.

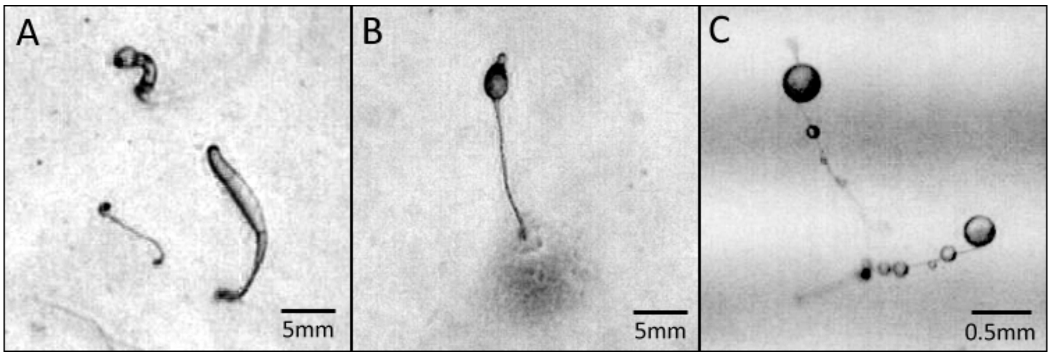

Figure 2. *Dictyostelium laterosorum.* **A.** early sorocarps. **B.** late sorocarp. **C.** mature fruiting bodies with sessile sori along length of stalk.

The sorocarps of *Polysphondylium pallidum* are described as erect, colorless with evenly spaced whorls that have 2-4 branches each (Fig. 3). Terminal sori are globose, white and larger than lateral sori which are found at the branches. Aggregates are radiate and compact. Spores are elliptical. *P. pallidum* prefer acidic soils (Cavender & Vadell, 2006) and is one of the most commonly isolated species in tropical forest soils.

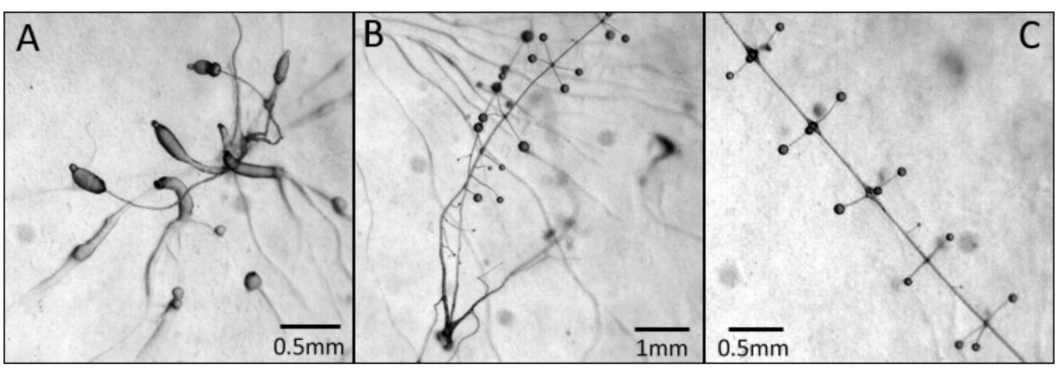

Figure 3. *Polysphondylium pallidum.* **A.** early clustered sorocarps. **B.** mature fruiting body with expanded base. **C.** mature fruiting body with whorled sororarp.

As of the latest listing of dictyostelids species in the Philippines (Dogma & Blancaver, 1965; Dogma, 1975; Cavender, 1976), only 11 species of dictyostelids have been reported from our country. *D. laterosorum* and *P. pallidum* are reported here for the first time from Subic Bay Forest Reserve in Zambales, Northern Philippines.

LITERATURE CITED

Cavender J. 1970. *Dictyostelium dimigraformum*, *Dictyostelium laterosorum* and *Acytostelium ellipticum*: New Acrasieae from the American Tropics. *Journal of General Microbiology* 62: 113-123.

Cavender J. 1976. Cellular Slime Molds of Southeast Asia. II. Occurrence and Distribution. *American Journal of Botany* 63 (1): 71-73.

Cavender J, Raper K. 1965a. The Acrasieae in Nature. I. Isolation. *American Journal of Botany* 52 (3): 294-296.

Cavender J, Raper K. 1965b. The Acrasieae in Nature. II. Forest Soil as a Primary Habitat. *American Journal of Botany* 52 (3): 297-302.

Cavender J, Vadell E. 2006. Cellular Slime Molds of Ohio. Ohio Biological Survey Bulletin New Series 16 (1): 55pp.

Dogma I Jr. 1975. Of Philippine Mycology and lower fungi. *Kalikasan Philippine Journal of Biology* 4: 69-105.

Dogma I Jr, Blancaver R. 1965. Cellular sliine molds from Philippine soil. *Philippine Phytopathology* 1: 41-52.

Fenchel T. 1987. *Ecology of Protozoa: the biology of free-living phagotrophic protists*. Science Tech Publishers, Madison, Wisconsin, USA.

Shim K, Chang N. 1998. Dictyostelid Cellular Slime Molds in Mt. Surak. *Korean Journal of Ecology* 21 (2): 157-161.

Stephenson S, Landolt J, Moore D. 1999. Protostelids, dictyostelids, and myxomycetes in the litter microhabitat of the Luquillo Experimental Forest, Puerto Rico. *Mycologia* 103 (2): 209-214.

Swanson A, Vadell E, Cavender J. 1999. Global Distribution of Forest Soil Dictyostelids. *Journal of Biogeography* 26 (1): 133-148 .