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The biodiversity of Dictyostelid cellular slime molds in rubber tree leaf litter in Southern Thailand

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Abstract

The occurrence of dictyostelid cellular slime molds were examined in rubber tree plantation habitats. Samples of leaf litter were collected from 10 rubber tree plantation fields in Nakhon Si Thammarat and Songkhla provinces and plated out for dictyostelid cellular slime molds. Ten species were identified, seven of them are *Dictyostelium* (*Dictyostelium dichotomum, D. macrocarpum*, *D. menorah*, *D. microsporum*, *D. minutum*, *D. mucoroides* and *D. rosarium*). The other three species belong to the genus *Polysphondylium* (*Polysphondylium multicystogenum, P. pallidum* and *P. violaceum*). The occurrence of dictyostelid slime molds in litter collected from use-of-herbicide areas tends to be of a much lower density (15.0–32.5 clones/1g leaf litter) than in non-use-of-herbicide areas (122.5–255.0 clones/1g leaf litter).

Key words – plant litter – rubber tree – slime mold

Introduction

The dictyostelid cellular slime molds are soil amoebae that can lead both unicellular (i.e. solitary) and multicellular (i.e. social) lives. Single amoebae feed on bacteria, grow and divide by mitosis. When starvation sets in, they stop dividing, aggregate and form an integrated multicellular unit, the slug, which exhibits division of labour. Eventually, some amoebae differentiate into dormant spores and form a coalescent mass, the sorus. The remaining amoebae form a dead cellular stalk and support the sorus; the whole is called a fruiting body (Bonner 1967). Dictyostelid cellular slime molds (dictyostelids) are common components of the soil/leaf litter interface, particularly in tropical, subtropical and temperate regions (Landolt & Wong 1998). They are usually present and often abundant in terrestrial ecosystems and apparently play a role in maintaining the natural balance that exists between bacteria and other microorganisms in the soil environment (Landolt et al. 2006a). Most of the life cycle of a dictyostelid is spent in a unicellular, amoeboid state, with cells feeding upon bacteria, protists, and microfungi. When such food supplies are abundant, slime mold myxamoebae proliferate by mitotic cell division. As the local bacterial food supply becomes relatively depleted, hundreds to thousands of myxamoebae, responding to chemotactic signals, aggregate and collectively differentiate as a sorocarp. The sorocorp consists of vacuolated cells, with cellulosic cell walls, which form a branched stipe with a cluster of spores at each stipe terminus (Landolt & Wong 1998, Bonner 2009).

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The diversity of dictyostelid cellular slime molds has been studied in soil in India (Cavender 1976, Cavender & Lakhanpal 1986), Switzerland (Traub et al. 1981), Nepal (Hagiwara 1982, 1990), Taiwan (Hagiwara et al. 1992), Korea (Shim & Chang 1996, 1998), Japan (Cavender & Kawabe 1989, Hagiwara 1989, 1995, Hagiwara & Kawakami 2000), the Philippines (Dogma & Blancaver 1965, Yulo & Cruz 2011), New Zealand (Cavender et al. 2002), Argentina (Vadell & Cavender 2007), west Africa (Kawakami & Hagiwara 2008) and the United States (Landolt et al. 2009, Cavender & Cavender 2013), in leaf litter in Hawaii (Landolt & Wong 1998) and in caves in West Virginia (Landolt et al. 1992), Arkansas, Missouri and Oklahoma (Landolt et al. 2005).

The rubber tree (*Hevea brasiliensis*) is the main cultivated plant of southern Thailand, and is grown throughout southern Thailand. It is a deciduous perennial tree with a major annual leaf shedding during December, leaf flush in January and flowering in February. Under rubber trees there is a great amount of decomposing rubber leaves. The humidity is high, especially in the rainy season. The study of dictyostelid cellular slime molds is less common in the tropical region and there are no reports about such molds under rubber plantation habitat conditions. The objective of this study is to carry out an extensive survey of dictyostelids in rubber tree leaf litter of southern Thailand.

Materials & Methods

The study was conducted at rubber plantations comprising rubber trees of the *H. brasiliensis* RRIM 600 variety in Nakhon Si Thammarat and Songkhla Provinces, both in southern Thailand. Rainfall varies greatly throughout the year due to the influence of monsoon winds. Nakhon Si Thammarat Province is located in the central southern area with a latitude of approximately 8°47'-9°00' N and longitude range of 99°97'-100°04' E. The province has two seasons: the dry season (many Thais call it “summer” because the weather is hot and dry) and the rainy season. The dry season is in February - April and the rainy season runs from May to January.

Songkhla Province is located on the eastern coast of the southern Thailand, between latitudes 6°17'-7°56' N and longitudes 100°01'-101°06' E. The province has two seasons, like Nakhon Si Thammarat, but the dry season is from February to the middle of July. Both areas experience the highest precipitation in November and the highest temperature in May. The total rainfall in 2013 was 2868. 6 mm in Nakhon Si Thammarat Province and 2793.6 mm in Songkhla Province and temperatures averaged about 27.94°C. Four out of seven areas in Nakhon Si Thammarat province were herbicide-use areas, the others are herbicide-non-use areas.

Collections of rubber tree leaf litter were made at 10 areas in rubber plantations. The 3 sub-samples were collected in each area. All samples (100g each) were placed in separate Ziplock plastic bags and taken to the laboratory for treatment within 24 hours. The collection was done according to methods similar to these of Landolt et al. (2009). 100 ml of sterile distilled water was mixed with 10 g samples of rubber tree leaf litter to obtain an initial suspension of 1:10. This mixture was blended with a blender for about 1-2 minutes to disperse the material and to suspend the cells of dictyostelid present. A 5.0 ml volume of this initial suspension was added to 0.8 ml of *Escherichia coli* and 6.7 ml of sterile water to produced a final dilution of 1:25 g of leaf litter per ml. One ml of suspension was placed on the surfaces of culture dishes containing hay infusion agar (10 replications). The culture dishes were maintained at room temperature (28-32°C). Each plate was examined under a stereomicroscope at least once a day for 15 days following the appearance of initial aggregations of amoebae or fructifications, and the location of each aggregation/ fructification (clone) was marked by pen on the bottom of the dish. Isolates were subculture to facilitate identification. The occurrence of each dictyostelid in 1 g of leaf litter was calculated.

Results

The data obtained from the cellular slime molds on rubber tree leaf litter in southern Thailand in this study are summarized in Table 1. Based on these data, dictyostelids would seem to be consistently present in the assemblages of microorganisms found on rubber tree leaf litter. A total 1197.5 clones have been examined for presence of dictyostelids. 10 species of dictyostelids were isolated in this preliminary survey. Seven of them are *Dictyostelium* (*D. dichotomum*, *D. macrocarpum, D. menorah*, *D. microsporum, D. minutum*, *D. mucoroides* and *D. rosarium*), while the other three species belong to the genus *Polysphondylium* (*P. multicystogenum*, *P. pallidum* and *P. violaceum*). It was found that *D. minutum* and *D. macrocarpum* were the dominant species observed in this study (Table 2).

The dictyostelid cellular slime molds were very diverse in sample S03 (9 species, 255.0 clones/1g leaf litter) and N01 (9 species, 222.5 clones/1g leaf litter), while the rest of the areas yielded 2-4 species, 15.0-240.0 clones/1g leaf litter. The amount of dictyostelid cellular slime molds in used-herbicide areas (N02, N03, N04 and N05) were very low (15.0-32.5 clones/1g leaf litter) compared to non-use-of-herbicide areas (S01, S02, C01 and C02) which tended to be high (122.5-240.0 clones/1g leaf litter). *D. minutum* and *D. macrocarpum* were the most abundant species, and the total number of clones isolated was 267.5 and 265.0 clones, respectively. Two species, *D. rosarium* and *P. multicystogenum*, were rarely isolated in this study (2.5 and 25.0 clones), while the rest species were commonly isolated.

List of species

D. dichotomum Vadell et Cavender., sorocarps solitary to clustered, sinose, erect to prone, dichotomously branched, bright yellow pigmentation, 0.6-2.5 mm in length. Sorophore architecture may show up to 4 successive forks. Sori are globose, bright yellow, 30-150 µm. Spore oblong-elliptical, yellow, with prominent polar to subpolar granules, mostly consolidate 5.1 x 2.9 µm. Large aggregations are of the violaceum type, irregular, bright yellow, with lobular secondary centers of aggregation and ample treams (Vadell & Cavender 2007).

D. macrocarpum Vadell et Cavender., sorocarps solitary to tightly clustered, 1.0-2.8 mm high, in general irregularly branched in aged cultures, sigmoid, prone. Sori are white-hyaline, globose, irregular, 80-200 µm diam. Spore elliptic to reniform, narrow, 4.0-6.5 x 2.0-3.0 µm. Aggregations are dome or mound-like at first, as a flattened globule, then becoming irregular and developing dendroid short regular streams, 1-2 mm in diam (Vadell & Cavender 2007).

D. menorah Vadell et Cavender., solitary to clustered, erect, generally branched, 350-550 µm high. Tips compound capitate (10-15 µm), sometimes unfinished and curved, surrounded by dense slime. Bases round to clavate, 20-25 µm in diam. Sori are globose, hyaline, 40-70 µm diam, with distinguishable spores. Spores oblong-elliptical, with some dispersed consolidated granules, mostly at poles, average 5.4 x 3.7 µm. Aggregation, small mounds, irregular in shape, average 200 µm (Vadell & Cavender 2007).

D. microsporum Hagiwara., sorocarps white, erect or inclined, usually unbranched, but occasionally bearing one or more lateral branches. Sorophores delicate, consisting of single tiers of cells except in the basal part, sinuous, usually 0.14-1.20 mm length or more. Sori are globose, usually 25-100 µm in diameter but sometimes up to 130 µm. Spore hyaline, ellipsoid, smooth, mostly 3.4-5.1 x 1.7-2.8 µm, having polar granules. Aggregation consist of well-developed radiate streams 0.5-2.0 mm in diameter that break up into several smaller aggregations prior to sorocarp formation (Raper 1984).

D. minutum Raper., this species is apparently cosmopolitan, one of the smallest species in the group. *D. minutum* has small sorocarps (0.10-1.67 mm long), spore elliptical to slightly reniform, short, average 5.0-6.0 x 2.5-3.0 µm, without polar granules, although some vesicles can be observed. Sori are colourless to milk white, globose to round-apiculate, 80 - 15 µm diam or larger. Aggregations are small, no longer than 200 µm, with irregular flattened and interconnected streams. It was found living together with *P. pallidum* (Cavender et al. 2002, Romeralo & Lado 2006).

D. mucoroides Rref., the main characteristics of *D. mucoroides* are its solitary erect, prone and prostrate, generally from 2.0-4.5 µm without branching. Sori are globose to citriform, 100-250 µm diam, white, becoming cream shed aged. Spores are elliptic, mostly 5.5-6.0 x 3.0-4.0 µm, without polar granules but with vesicles and small. Aggregations are typically of the “mucoroides” type (Raper 1984, Hagiwara 1989).

Table 1 A summary of the data obtained in the present study on rubber tree leaf litter (*H. brasiliensis*) for dictyostelids from 10 areas in Nakhon Si Thammarat and Songkhla provinces (an average from 10 replications, in each case).

Study site	Code	Plantation management	Located		Clones/1g leaf litter
			Lat. (S)	Long.(E)	
Nakhon Si Thammarat Province					577.5
Thung Yai, A	N01	non-use-of-herbicide	8°17'	99°22'	222.5
Thung Yai, B	N02	use-of-herbicide	8°17'	99°22'	15.0
Thung Song, A	N03	use-of-herbicide	8°09'	99°40'	30.0
Thung Song, B	N04	use-of-herbicide	8°09'	99°40'	27.5
Nabon	N05	use-of-herbicide	8°15'	99°35'	32.5
Cha-Uat	C01	non-use-of-herbicide	7°57'	99°59'	122.5
Chian Yai	C02	non-use-of-herbicide	8°10'	100°08'	127.5
Songkhla Province					620
Khlong Hoi Khong	S01	non-use-of-herbicide	6°53'	100°23'	240.0
Hat Yai, A	S02	non-use-of-herbicide	7°00'	100°27'	125.0
Hat Yai, B	S03	non-use-of-herbicide	7°00'	100°27'	255.0
	Total				1197.5

D. rosarium Raper and Cavender., sorocarps solitary to clustered, prone to pendant, 1.5-4.5 mm long with a variable number of sessile sori (1-15, but commonly 3-9). Terminal sori are globose, hyaline, 80-220 µm diam. Lateral sori are 20-80 µm diam. Spore are round, very regular, with no granules average 4.5-5.5 µm, sorogens are large (300-800 µm). Aggregation is distinctive and should be designated as being of the "rosarium" type (Cavender et al. 2002).

P. multicystogenum S. Kawakami et H. Hagiwara., a new dictyostelid was recovered from soil in Sierra Leone, West Africa by Kawakami& Hagiwara (2008). Sorocarps usually solitary or sometimes clustered, erect or inclined, sometimes prostrate, phototropic, with 1-11 nodes, with 2-8 branches per whorl and often with irregular branches. Sorophores are colorless, sinuous, mostly 1.3-6.6 mm long. Sori are white, globose; terminal sori mostly 72-109 µm diam. Spore hyaline is oblong to elliptical, usually 1.6-2.0 times longer than width, mostly 5.0-6.0 x 2.9-3.5 µm, with unconsolidated polar granules and sometimes with irregular granules. Pseudoplasmodia are radial or fan-shaped, centralized (Kawakami & Hagiwara 2008).

P. pallidum Olive., the main distinguishing characteristics of this species are the solitary sorocarps, the number of whorls (1-11) with branches between two and six, sorocarps without lengthened terminal segments, not photophopic, colorless, the spores with unconsolidated polar granules and the radial aggregation (Romeralo & Lado 2006).

P. violaceum Brefeld., this is a common and widespread inhabitant of forest soils and litter. Sorocarps are solitary to loosely clustered, 2-5 mm in height, erect or semierect, with 3-8 whorls of violaceous pigmented small lateral sori, nodes are regularly spaced. Sorophores with vinaceous pigment, elongated (4-15 mm) in one sided illumination and may not develop whorls. Spores are elliptical to oblong, vinaceous to hyaline, with consolidated granules, mostly 5.5-7.0 x 3.0-4.0 µm. Aggregations are of the "violaceum" type, radiate and are reaching 2 mm or more in diameter (Cavender et al. 2002).

Discussion

There are few studies on dictyostelid cellular slime molds communities occurring on plant litter in tropical forests. However, some studies have been carried in northern Thailand (Landolt et al. 2008a, b). This is the first reported study of dictyostelid cellular slime molds in rubber plantation from southern Thailand. A total of 10 species from 2 genera were isolated during this study, *Dictyostelium* (7 species) and *Polysphondylium* (3 species). *D. minutum* and *D. macrocarpum* were the dominant species found in this study. Cavender & Raper (1965) reported that *D. minutum* is the most characteristic species, often occurring in the deciduous forest, where there is a heavy accumulation of undecomposed or partially decomposed leaf litter. According to Hagiwara (1990) studies of dictyostelid slime molds in the Langtang Valley, Nepal, showed that *D. minutum* often occurred in the subalpine deciduous forest. Romeralo & Lado (2006) reported that *D. minutum* has appeared in almost all the soil from Mediterranean forest and is living together with members of genus *Polysphondylium*, such as *P. pallidum.* However, *D. minutum* has rarely been found in subalpine conifer forest (Traub et al. 1981, Hagiwara 1982, Cavender 1983, Cavender & Lakhanpal 1986).

In this study, *D. dichotomum, D. macrocarpum, D. menorah, D. microsporum, D. mucoroides* and *P. pallidum* were the most common species of dictyostelid cellular slime molds found on rubber tree leaf litter. *D. dichotomum, D. macrocarpum* and *D. menorah* were recently recovered from soils in Atlantic subtropical rain forest in northeastern Misiones province, Argentina by Vadell & Cavender (2007). Hagiwara (1989) reported that *P. pallidum* was found in many localities in America, Africa, Europe and Asia. In tropical forest, Stepheson & Landolt (1998) studied canopy soil in Puerto Rico and showed that *D. pallidum* tends to be found in regions with a tropical or subtropical climate and tropical forest (At least, that is the case in Puerto Rico). Cavender (1973) was of the view that *P. pallidum* and *D. mucoroides* are the one of the few cellular slime molds that seem to be indifferent to climatic conditions. Consequently, they are two of the most widely distributed cellular slime molds of the forest types located at lower elevations and also in two of the higher elevation forest types. Romeralo & Lado (2006) reported that *P. violaceum* and *D. mucoroides* were the most common species in the south Mediterranean area, and appeared in almost all of the substrates such as under *Acer* spp., *Fagus* spp. and *Pinus* spp. coexisting with many other species of cellular slime mold.

Twenty five clones of *D. rosarium* were found in two study areas (S03 in Songkhla province and N01 in Nakhon Si Thammarat province). These species appear to have an unusual and rather restricted distribution (Raper 1984). They have been found in north America occasionally in dry/saline soils above ground (Benson & Mahoney 1977), in caves in west Virginia, Alabama, Arkansas and Missouri (Landolt et al. 1992, 2006b, Cavender et al. 2002). In this study, it is interesting that *P. multicystogenum* was found in only one area in Songkhla province (S03). The average number of clones obtained from 1g of leaf litter was 2.5 clones which is very rare species isolation. *P. multicystogenum* was recovered from Sierra Leone, west Africa by Kawakami & Hagiwara (2008).

Cavender (1976) reported finding that six species of dictyostelids ranked in highest importance in both tropical American and tropical Asian forests. Three of these six species (*D. mucoroides, P. pallidum* and *P. violaceum*) were recovered from rubber tree leaf litter in Thailand. The overall degree of species richness and densities of dictyostelids in rubber tree leaf litter in Thailand is actually relatively high for isolated dictyostelids in tropical and subtropical zones; fourteen species were identified by Cavender (1970) in Trinidad and Tobago, and in Lambuan on the island of Panay in the Philippines; nine species were identified by Cavender (1976) and four species recovered in Luzon and Mindanao by Dogma & Blancaver (1965).

In four study areas in Nakhon Si Thammarat province (N05, N03, N04 and N02), the dictyostelid cellular slime molds tend to be of low density and distribution (32.5, 30.0, 27.5 and 15.0 clones/1g leaf litter). This would appear to be a result of the plant communities being in rubber plantations which were use-of-pesticide areas such as areas where herbicide was used for weed control with effects on cellular slime molds. In other areas (S03, S01, N01, C02, S02 and C01) where the plant communities in rubber plantation were not disturbed (herbicide was not used), the dictyostelid cellular slime molds tended to be of high density (255.0, 240.0, 222.5, 127.5, 125.0 and 122.5 clones/1g leaf litter). Only 3 species, *D. dichotomum, D. macrocarpum* and *D. mucoroides* were found both in areas where herbicide was used and in areas herbicide was not used, but the number of clones is rather low. Conversely, 7 species, *D. menorah, D. microsporum, D. minutum, D. rosarium, P. multicystogenum, P. pallidum* and *P. violaceum* were not found in herbicide-use areas. Pailet & Satre (2010) studied the biodiversity of dictyostelids in soil in mountain forests in France, to compare the diversity in unmanaged and managed plots, and showed that soil disturbance by a management regime on managed plots resulted in lower diversity than in

Table 2 The occurrence of dictyostelid cellular slime molds isolated from rubber tree leaf litter. Data are numbers of clones/1g leaf litter (an average from 10 replications, in each case).

Species	Study areas										Total no. of clones
	Nakhon Si Thammarat Province							Songkhla Province			
	N01	**N02**	**N03**	**N04**	**N05**	**C01**	**C02**	**S01**	**S02**	**S03**	
D. dichotomum	20.0	7.5	12.5	12.5	20.0						72.5
D. macrocarpum	52.5	7.5	12.5	12.5	12.5		72.5	72.5		22.5	265.0
D. menorah	20.0							52.5		55.0	127.5
D. microsporum	20.0									37.5	57.5
D. minutum	25.0					27.5	55.0	87.5	47.5	25.0	267.5
D. mucoroides	32.5		5.0	2.5		15.0			32.5	47.5	135.0
D. rosarium	20.0									5.0	25.0
P. multicystogenum										2.5	2.5
P. pallidum	17.5					80.0			45.0	32.5	175.0
P. violaceum	15.0							27.5		27.5	70.0
No. of species	9	2	3	3	2	3	2	4	3	9	
Clones/1g leaf litter	222.5	15.0	30.0	27.5	32.5	122.5	127.5	240.0	125.0	255.0	1197.5

unmanaged forest.

Some species of dictyostelid cellular slime molds are associated with a variety of kinds of soil. Romeralo & Lado (2006) explained that the microhabitat represented by the mantle of organic material at the base of a vascular epiphyte is limited in extent and spatially isolated, especially soil in forest types where such epiphytes are uncommon. Our data suggest that the presence of cellular slime molds in this microhabitat is a rare occurrence especially in leaf litter in rubber plantations where herbicide was used (15.0-32.5 clones/1g leaf litter). Lesica & Antibus (1990) reported that the roots of tropical epiphytes tend to be characterized by very low levels of colonization by vesicular-arbuscular mycorrhizal fungi, even though these fungi are abundant in terrestrial communities. They attributed this to an inability of the fungi to disperse via the air to isolated soil habitats. Cellular slime molds also appear to have a rather limited potential for dispersal. These factors affected the apparent widespread occurrence of cellular slime molds because the spores produced by cellular slime molds are embedded in a mucilaginous matrix which dries and hardens. As a result, the spores stand little chance of being dispersed by wind (Cavender 1973, Olive 1975). However, it has been clearly demonstrated that viable dictyostelid spores may be dispersed over great distances by migratory songbirds (Suthers 1985) and over shorter distances by salamanders, rodents and bats (Stephenson & Landolt 1992) and within a given microsite by soil invertebrates (Huss 1989). In the rubber plantation habitats used for this study, a variety of insects, plant communities, arthropod, snails were observed on the plantation floor. It seems very likely that all of these organisms could serve as vectors for the spores of the cellular slime mold. Many factors affected the distribution and density of dictyostelid cellular slime molds such as abiotic factors (moisture, organics, pH, temperature, soil quality and altitudes or elevation) and biotic factors (prey-predator and other inter-species interactions) (Stephenson 1988, Chang et al. 1996, Shim & Chang 1996, Swanson et al. 1999, Bonner & Lamont 2005, Paillet & Satre 2010). The rubber tree leaf litter can support populations of cellular slime molds and this is probably not surprising given that rubber tree leaf litter is highly variable for many of the biologically important nutrients (e.g. N,P, K and Ca). Besides, under rubber trees there is high humidity and large amounts of organic matter. Cellular slime molds are not the only organisms that occur in rubber tree leaf litter microhabitats; there were also nematodes and these were present in a number of the primary isolation plates prepared with samples of rubber tree leaf litter, and myxomycetes plasmodia appeared in a few plates.

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