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Dictyostelids from aerial "canopy soil" microhabitats

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ARTICLE INFO

Article history:
Received 11 May 2010
Revision received 18 December 2010
Accepted 5 January 2011
Available online 5 March 2011
Corresponding editor:
Martin Schnittler

Keywords:
Dictyostelids
Ecology
Forests
Microhabitats
Vectors

ABSTRACT

The leaf litter decomposition zone of forest soils is generally considered as the primary microhabitat for dictyostelid cellular slime molds (dictyostelids), but these organisms also occur in other types of soils and are sometimes coprophilous. The occurrence of dictyostelids in aerial microhabitats has received relatively little study. However, they can be surprisingly abundant in the mantle of dead organic matter (literally a "canopy soil") often found at the bases of epiphytes that grow on the larger branches and trunks of trees in moist temperate and tropical forests. More than 400 samples collected from the canopy soil microhabitat in 11 localities in several different regions of the world yielded at least 37 species of dictyostelids. Some of the species recovered from canopy soil have been described as new to science, and three of these are not yet known from ground soil.

Introduction

Dictyostelid cellular slime molds (dictyostelids) are single-celled, eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems. These organisms represent a normal component of the microflora in soils and apparently play a role in maintaining the natural balance that exists between bacteria and other microorganisms in the soil environment (Raper 1984). The primary habitat for dictyostelids is the leaf litter decomposition zone of forest soils (Cavender *et al.* 2005). However, they are known to occur in other types of soils. Among these are soils of cultivated regions (Agnihothrudu 1956), grasslands (Smith & Keeling 1968), deserts (Benson & Mahoney 1977), and both alpine (Cavender 1983) and arctic (Cavender 1978; Stephenson *et al.* 1991) tundra. In addition, dictyostelids can be surprisingly abundant in the soils found in caves (Landolt *et al.* 1992, 2006) and are sometimes coprophilous (Raper 1984).

In a previous paper (Stephenson & Landolt 1998), we reported that dictyostelids can sometimes occur in the mantle of dead organic material (literally a "canopy soil") associated with the epiphytes that occur on the branches and, to a lesser extent, the trunks of tropical trees. This dead organic matter is derived from decaying epiphytes, partially decomposed tree bark, insect frass and intercepted litter. Such canopy soil microhabitats are known to represent an important subsystem of tropical forests (Nadkarni & Matelson 1991; Lowman & Nadkarni 1995), but they remain understudied for many groups of microorganisms. In the study by Stephenson & Landolt (1998), dictyostelids were isolated from 18 of 50 (36 %) samples of canopy soil collected from five study sites located in the tropical forests of the Luquillo Mountains in eastern Puerto Rico. Four different species (*Dictyostelium firmibasis*, *Dictyostelium purpureum*, *Polysphondylium pallidum* and a *Dictyostelium* that could not be assigned to any described taxon) were recovered. The impetus for this study was

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doi:10.1016/j.funeco.2011.01.002

a concurrent sampling effort (Stephenson *et al.* 1998) directed at the dictyostelids, myxomycetes and protostelids associated with the litter layer on the forest floor but which also included several samples of canopy soil that unexpectedly yielded dictyostelids. Since this initial study, the occurrence of dictyostelids in canopy soil microhabitats has been examined as one component of other surveys for both dictyostelids and (especially) myxomycetes carried out in the context of two major biodiversity inventory projects funded by grants from the National Science Foundation. The purpose of this paper is to summarize the body of data obtained during the course of other surveys in which it was possible to obtain at least some samples of canopy soil.

Materials and methods

Between 1996 and 2008, more than 400 samples (each approximately 20–30 g wet weight) of canopy soil for isolation of dictyostelids were collected in 11 localities in several different regions of the world (Table 1). In some instances, a particular locality (e.g., Ascension Island, Cuba, and the Dominican Republic) was represented by only a single collecting site, whereas in other localities (e.g., Australia, Costa Rica, and the Pacific Northwest of the USA) samples were obtained from a series of collecting sites. Localities from which samples were collected encompassed three different continents and several islands.

In most cases, the samples referred to above were collected as part of a more extensive sampling effort (e.g., Black *et al.* 2004; Stephenson *et al.* 2004; Camino *et al.* 2008; Landolt *et al.* 2008b) in which the primary emphasis was on myxomycetes. Moreover, some sets of samples (e.g., those from the western United States, the Dominican Republic, Belize and Honduras) were collected by one of our colleagues or one of Stephenson's students. The actual collecting sites included examples in a number of different types of tropical forests (e.g., coastal rain forests, lowland rain forests, montane rain forests and cloud forests) as well as other types of forests (e.g., temperate coastal rain forests and montane southern beech forests). In northern Queensland in Australia, where we had access to a canopy crane, some samples were collected at a height of more than 40 m above the forest floor. All study sites were referenced to geographic location through the use of the NAVSTAR Global Positioning System (GPS), with latitude and longitude determined by means of a portable GPS unit.

All samples were stored in sterile plastic bags and returned to the laboratory. In some instances, this involved mailing sample material back to the USA, which often required 10 d or even longer. Presumably, some reduction in the number of viable propagules of dictyostelids (i.e., spores, microcysts or active amoebae) would have taken place during this time. However, because of United States Department of Agriculture import restrictions, this situation could not have been avoided. In the laboratory, samples were processed following the procedures described by Cavender & Raper (1965). Each sample of canopy soil was weighed and enough sterile distilled water added to obtain an initial suspension of 1:10. This mixture was shaken to disperse the material and to suspend the cells of dictyostelids present. A 5.0 ml volume of this initial suspension was added to 7.5 ml of sterile distilled water to create a 1:25 final dilution. Aliquots (each 0.5 ml) of the 1:25 dilution were added to the surface of each of two or three 100 by 15 mm plastic disposable Petri dishes containing hay infusion agar (Raper 1984). Approximately 0.4 ml of a heavy suspension of *Escherichia coli* was added to each Petri plate, and the plates incubated under diffuse light at 22–25 °C. Each inoculated plate was examined carefully at least once a day following the appearance of initial aggregations of amoebae or fruitings that had developed from the propagules present in the sample material, and each aggregation/fruiting ("clone") was marked. Numbers of clones assigned to a particular taxon were calculated, and these figures were used to determine both density (number of clones of dictyostelids per gram of sample material) and frequency of occurrence (proportion of all samples in which a given taxon was recorded) for the set of samples from a particular locality. When necessary, isolates of particular clones were subcultured to facilitate identification. Later, some of these were conserved in the collection of dictyostelids maintained by the second author at Shepherd University.

Results

Samples collected in the present study yielded at least 37 species of dictyostelids (Table 2). This total included 34 species and one variety that could be identified with a high degree of

Table 1 – Localities where samples of "canopy soil" for isolation of dictyostelids were collected

Locality	No. of samples	Latitude/longitude	Forest type(s)
Australia	38	16°06′–17°12′S, 145°26′–145°40′E	Mesophyll vine forest
Cuba	10	21°53′N, 79°36′W	Wet montane rain forest
Western United States	68	44°11′–44°28′N, 122°23′–123°34′W	Temperate coastal rain forest
Costa Rica	70	09°45′–10°56′N, 82°49′–85°27′W	Lowland tropical forest and cloud forest
Dominican Republic	5	19°08′N, 69°49′W	Coastal rain forest
Ecuador	47	00°05′N, 78°37′W	Cloud forest
New Zealand	5	44°48′S, 108°06′E	Southern beech (*Nothofagus*) forest
Puerto Rico	95	18°20′N, 65°49′W	Montane tropical forest
Belize	60	15°33′N, 88°42′W	Lowland evergreen tropical rain forest
Honduras	10	14°50′N, 89°08′W	Lowland tropical rain forest
Ascension Island	5	07°57′S, 14°21′W	Planted *Pandanus utilis* below summit of Green Mountain