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DICTYOSTELID CELLULAR SLIME MOLDS FROM CAVES

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*Dictyostelid cellular slime molds associated with caves in Alabama, Arkansas, Indiana, Missouri, New York, Oklahoma, South Carolina, Tennessee, West Virginia, Puerto Rico, and San Salvador in the Bahamas were investigated during the period of 1990–2005. Samples of soil material collected from more than 100 caves were examined using standard methods for isolating dictyostelids. At least 17 species were recovered, along with a number of isolates that could not be identified completely. Four cosmopolitan species (*Dictyostelium sphaerocephalum*,* D. mucoroides*,* D. giganteum *and* Polysphondylium violaceum*) and one species (*D. rosarium*) with a more restricted distribution were each recorded from more than 25 different caves, but three other species were present in more than 20 caves. The data generated in the present study were supplemented with all known published and unpublished records of dictyostelids from caves in an effort to summarize what is known about their occurrence in this habitat.*

Introduction

Dictyostelid cellular slime molds (dictyostelids) are single-celled, eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems (Raper, 1984). 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. For most of their life cycle, dictyostelids exist as independent, amoeboid cells (myxamoebae) that feed upon bacteria, grow, and multiply by binary fission. When the available food supply within a given microsite becomes depleted, numerous myxamoebae aggregate to form a structure called a pseudoplasmodium, within which each cell maintains its individual integrity. The pseudoplasmodium then produces one or more fruiting bodies (sorocarps) bearing spores. Dictyostelid fruiting bodies are microscopic and rarely observed except in laboratory culture. Under favorable conditions, the spores germinate to release myxamoebae, and the life cycle begins anew. Dictyostelids are most abundant in the surface humus layer of forest soils, where populations of bacteria are the highest and microenvironmental conditions appear to be the most suitable for dictyostelid growth and development (Raper, 1984).

While the primary habitat for dictyostelid cellular slime molds (or dictyostelids) is the leaf litter decomposition zone of forest soils, these organisms are known to occur in other types of soils. Among these are soils of cultivated regions (Agnihothrudu, 1956), grasslands (Smith and Keeling, 1968), deserts (Benson and Mahoney, 1977), and both alpine (Cavender, 1973) and arctic (Cavender, 1978; Stephenson *et al*., 1991) tundra. In addition, dictyostelids have been reported from the layer of soil-like material (canopy soil) associated with the epiphytes that occur on the branches and trunks of tropical trees (Stephenson and Landolt, 1998). Dictyostelids also occur on dung and were once thought to be primarily coprophilous (Raper, 1984). However, perhaps the most unusual microhabitat for dictyostelids is the soil material found in caves. Few studies have considered the dictyostelids associated with caves. In what apparently represents the first published report of dictyostelids in caves, Orpurt (1964) reported two species (*Dictyostelium mucoroides* and *Polysphondylium pallidum*) from a cave located on Eleuthera Island in the Bahamas. Later, Waddell (1982) reported eight species from Blanchard Springs Cavern in Arkansas. One of these (*Dictyostelium caveatum*) was new to science. In the most extensive study to date, Landolt *et al*. (1992) investigated 23 caves in West Virginia. Nine species of dictyostelids were recovered, and three of these were present in at least 10 different caves. One of these three species (*Dictyostelium rosarium*) was of particular interest, since it had not been recorded from soil samples collected from above-ground sites in an earlier study of the distribution and ecology of dictyostelids in West Virginia (Landolt and Stephenson, 1990). In general, based on available data, the distribution of dictyostelids in caves appears to be rather patchy, but in the microsites where they do occur, these organisms can exhibit surprisingly high levels of abundance and diversity.

The objective of the present study was to extend the earlier investigation of dictyostelids in West Virginia caves (Landolt *et al*., 1992) to caves at a number of other localities, with particular emphasis placed on caves in the Ozark region of Arkansas, Missouri and Oklahoma (Landolt *et al*., 2005). In addition, these data were supplemented with all known published (Waddell, 1982; Landolt and Stihler, 1998; Reeves *et al*., 2000; Reeves, 2001; Nieves-Rivera, 2003) and unpublished records of dictyostelids from caves in an effort to summarize what is known about their occurrence in this habitat.

Material and methods

The caves considered in the present study are located in Alabama, Arkansas, Indiana, Missouri, New York, Oklahoma, South Carolina, Tennessee, West Virginia, Puerto Rico, and San Salvador in the Bahamas. All of these were sampled during the period of 1990 to 2005. Samples of cave substrate material, from the floor and from ledges, were collected from arbitrarily selected locations within each cave. Most samples were collected in conjunction with other cave survey work. In general, sample sites within a cave were chosen to represent the variety of different substrates available in that cave. If present, samples containing guano, plant debris or detritus were included along with mineral substrate samples. Depending upon the particular cave, samples ranged in texture from powdery dry dust or gravel to very wet clay mud. Samples were stored in sterile plastic bags, returned to the laboratory and processed as soon as possible following collection, using procedures similar to those described by Cavender and Raper (1965). In this procedure, 5–10 g of sample are suspended in sterile, distilled water to make a soil dilution ratio of either 1:10 or 1:25. An aliquot of the suspension (containing 0.02 g soil) is added to each of 2–3 plastic culture dishes containing a phosphate buffered (pH 6.0), filtered hay infusion agar. This medium is prepared by autoclaving 10–20 g of dry hay/L distilled water, filtering and adding 1.5 g KH_2PO_4, 0.62 g $Na_2HPO_4 \bullet 7H_2O$, 15 g agar/L filtrate. Each dish received approximately 0.3 mL of *Escherichia coli*, and culture plates were incubated under diffuse light at 10–25 °C. Each plate was carefully examined at least once a day for several days following appearance of initial aggregations and the location of each aggregate colony marked. When necessary, particular isolates were subcultured to facilitate identification. Nomenclature used herein follows that of Raper (1984).

Results

The data obtained from the caves examined in the present study along with other published and unpublished records of

Table 1. Summary data (obtained in the present study or reported in the literature) on caves sampled for dictyostelids. The figure given for the Ozarks represents the combined total for Arkansas, Missouri and Oklahoma.

Region	No. of caves investigated	No. of caves with dictyostelids	Percentage (%)	No. of species recovered
West Virginia [a, c]	61	58	95	12
Arkansas [b, c]	17	6	35	5
Missouri [c]	15	11	73	7
Oklahoma [c]	3	3	100	4
Ozarks (Subtotal)	35	20	57	8
Georgia [d]	2	2	100	6
South Carolina [e]	1	1	100	4
New York [c]	2	1	50	1
Indiana [c]	2	2	100	4
Tennessee [c]	3	3	100	6
Alabama [c]	4	4	100	8
Puerto Rico [c, g]	8	6	75	9
Bahamas [c, h]	5	5	100	5
Total	123	102	83	18

[a] Landolt *et al*., 1992
[b] Waddell, 1982
[c] Present study
[d] Reeves *et al*., 2000
[e] Reeves, 2001
[f] Davidson, unpublished data
[g] Nieves-Rivera, 2003, and unpublished data
[h] Landolt and Stihler, 1998

Table 2. Occurrence of dictyostelids in caves considered in the present study. The figure given for the Ozarks represents the combined total for Arkansas, Missouri and Oklahoma.

Region	Dsp[a]	Dmu	Dro	Dgi	Dmi	Dau	Ddi	Dpu	Dca	Dma	Dte	Dci	Dpo	Dvi	Pvi	Ppa	Pca	Pte	Total Species
West Virginia	41	26	16	16	17	20	6	5							6	3	1	1	12
Arkansas	2	2	3	2			1	2	1						4	2			9
Missouri	1	3	7	2				2							5	4			7
Oklahoma	1					1									1	1			4
Ozarks (Subtotal)	4	5	10	4		1	1	4	1						10	7			10
Georgia	1	1		1		2		1							2				6
South Carolina	1	1													1	1			4
New York	1																		1
Indiana	2	1					1								1				4
Tennessee		2		2	3	1		2								1			6
Alabama		4	2	3	1	1		2							4	3			8
Puerto Rico		1		2		2		3		2	1	1			1	5			9
Bahamas								5					2	2	3			1	5
Total Records	50	41	28	28	21	27	8	22	1	2	1	1	2	2	28	20	1	2	

Note: Total records refers to the number of caves from which the species in question has been recorded.

[a] Dsp = *Dictyostelium sphaerocephalum*, Dmu = *D. mucroroides*, Dro = *D. rosarium*, Dgi = *D. giganteum*, Dmi = *D. minutum*, Dau = *D. aureo-stipes*, Ddi = *D. discoideum*, Dpu = *D. purpureum*, Dca = *D. caveatum*, Dma = *D. macrocephalum*, Dte = *D. tenue*, Dci = *D. citrinum*, Dpo = *D. polycephalum*, Dvi = *D. vinaceo-fuscum*, Pvi = *Polysphondylium violaceum*, Ppa = *P. pallidium*, Pca = *P. candidum* and Pte = *P. tenuissimum*.

dictyostelids in caves are summarized in Table 1. Based on these data, dictyostelids would seem to be consistently present in the assemblages of microorganisms found in caves, with 102 of the 123 (83%) caves known to have been examined for the presence of dictyostelids yielding at least one species. In West Virginia, the region for which the most data exist, dictyostelids were recovered from 95% of the 61 caves investigated. Most records of dictyostelids in caves are from temperate North America, but these organisms also were recovered from 11 of 13 (85%) caves surveyed in Puerto Rico and the Bahamas.

At least 17 species of dictyostelids were isolated from samples of cave soil collected during the course of the present study (Table 2), along with a number of isolates that could not be identified completely. *Dictyostelium giganteum*, *D. mucoroides*, *D. rosarium, D. sphaerocephalum* and *Polysphondylium violaceum* were the most common species, and each was recorded from more than 25 different caves. Three other species (*D. aureo-stipes*, *D. purpureum* and *P. pallidum*) were recovered from more than 20 caves. Most of the other species recovered from caves were much less common, and several *(e.g.*, *D. citrinum*, *D. macrocephalum* and *D. polycephalum*) were recorded from only a single cave. Just one species (*D. caveatum*) reported in the literature from caves was not encountered in the present study. This species, recovered by Waddell (1982) from a cave in Arkansas, has not been reported since, either from caves or from aboveground sites.

DISCUSSION

The considerable body of data compiled for dictyostelids in caves in eastern North America indicates that these organisms should be considered part of the common microflora found in cave habitats. As a general observation, the species of dictyostelids that occur in caves are much the same as those most likely to be recovered from samples of above-ground soil (especially forest soil) in the general region of the cave in question. For example, with a single exception, all of species now known from more than 25 caves are generally considered to be among the most common inhabitants of forest soils (Raper, 1984; Swanson *et al*., 1999). Interestingly, samples from caves in subtropical regions (Puerto Rico and the Bahamas in the present study) yielded species of dictyostelids (*e.g.*, *Dictyostelium citrinum* and *D. macrocephalum*) thought to have distributions centered in tropical/subtropical regions of the world (Swanson *et al*., 1999). As such, the absence of these species in caves located in temperate regions, which was the case for the vast majority of caves sampled in the present study, is not surprising.

Dictyostelium rosarium appears to be the one major exception to this general pattern. This species appears to have an unusual and rather restricted distribution in nature (Raper, 1984). It has been found in North America only occasionally in dry/saline soils above ground (Benson and Mahoney, 1977) but was reported to occur with a surprising degree of regularity in caves in West Virginia by Landolt *et al*. (1992). In the present study, *D. rosarium* was commonly recorded from caves, including additional caves in West Virginia as well as others sampled in Alabama, Arkansas, and Missouri. The relative abundance of *D. rosarium* in caves in at least temperate North America is particularly noteworthy because the species appears to be rare outside of North America. For example, only a single isolate is known from the entire Southern Hemisphere (Cavender *et al*., 2002).

Three genera are currently recognized for the dictyostelids. While two of these (*Dictyostelium* with 14 species and *Polysphondylium* with four species) appear to be well represented in cave habitats, there are apparently no records of any member of the third genus (*Acytostelium*) from cave habitats. Species of *Acytostelium* are generally smaller and more delicate than members of the other genera, and it is possible that such forms simply do not survive well in caves, for reasons that are not yet known. Evidence for such a conclusion is suggested by the apparent absence of *D. lacteum* from caves. This species is common in forest soils throughout eastern North America but also is smaller and apparently more delicate than the majority of dictyostelids known from caves.

Unlike many microorganisms, dictyostelids produce spores that appear to have a rather limited potential for dispersal. In the dictyostelid life cycle, the unicellular amoeboid cells that represent the vegetative stage aggregate and form a structure called a pseudoplasmodium, which then gives rise to one or more fruiting structures (sorocarps), each bearing one to several masses of spores (sori). Since the spores are embedded in a mucilaginous matrix that dries and hardens, they stand little chance of being dispersed by wind (Cavender, 1973; Olive, 1976). It has been demonstrated (Suthers, 1985; Stephenson and Landolt, 1992) that various animals, ranging from invertebrates to amphibians, small mammals, and birds are capable of dispersing the spores of dictyostelids by means of ingestion-defecation. For example, Stephenson and Landolt (1992) isolated dictyostelids from the fecal material of bats and suggested that the latter may introduce dictyostelids to caves. In the present study, virtually all of the caves sampled for dictyostelids were known to support populations of bats. Indeed, actual collecting of sample material was carried out in the context of studies related to monitoring the bats present in a particular cave. It is very likely that organisms other than bats can serve as vectors for dictyostelid spores. Cave crickets (*Ceuthophilus gracilipes* [Halderman]) collected from one cave in Arkansas have been demonstrated to carry dictyostelid spores on the surface of their body (Stephenson and Slay, unpub. data). Since these crickets forage in the litter layer of forests outside of the cave, it is possible that they could introduce dictyostelid spores into the cave in addition to transporting spores from one place to another within a given cave. This aspect of the dictyostelid ecology warrants additional study. A few of the caves included in the survey are visited frequently by humans, but the great majority of the caves are sparsely visited by people because of such factors as small size, difficult access or restricted access for the protection of bat colonies.

Since dictyostelids depend upon a variety of aerobic bacteria for food, almost certainly the guano produced by bats represents a factor of considerable importance, although dictyostelids were rarely recovered directly from guano piles. Limited data obtained for a series of five samples obtained from the center of a guano pile outward suggest that dictyostelids are most abundant in the zone just outside the actual pile (Stephenson *et al*., unpub. data). As such, the question of whether bats introduce dictyostelids to caves still remains problematic, but it seems likely that bats are largely responsible for providing sufficient organic material to permit dictyostelids to survive in caves. Except for deposits of guano, organic material subject to bacterial decomposition is usually sparse in caves (Dickson and Kirk, 1976). Some caves may receive additional organic input as a result of surface water flow into the cave, and in one or two caves included in this study, cave rodent activity was specifically noted by sample collectors.

In summary, although caves might seem to represent an unusual habitat for dictyostelids, they do provide environmental conditions (*i.e.*, high humidity along with stable temperatures) that are reasonably suitable for these organisms, as indicated by the data presented in this paper.

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