Short Notes

BACTERIAL AND YEAST FOOD PREFERENCES OF CELLULAR SLIME MOLDS (DICTYOSTELIDS) ISOLATED FROM LUBANG ISLAND, OCCIDENTAL MINDORO, PHILIPPINES

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ABSTRACT

Cellular slime molds (dictyostelids) are single-celled, phagotropic micropredators in soil. Often, these organisms are grown in the laboratory with *Escherichia coli* as the food bacterium. In this study, we evaluated the feeding preferences of eight species of dictyostelids previously isolated from Lubang Island, Occidental Mindoro, Philippines. Our results showed that the isolated dictyostelids preferred gram-negative bacteria over gram-positive bacteria and yeasts. *E. coli* remained the food of choice by the most of the isolated cellular slime molds. Our study is the first attempt to evaluate the feeding preferences of locally isolated dictyostelids.

KEYWORDS: *bacteriovores, soil micropredators, food bacteria/yeasts, feeding preferences*

Cellular slime molds or dictyostelids are single-celled, phagotrophic micropredators that feed on bacteria. They are commonly found as members of soil microbial communities in the humus layer of forest soils in most terrestrial ecosystems around the world (Raper, 1984; Cavender & Raper, 1965b, 1965c; Swanson *et al*., 2002). Cellular slime molds belong to three genera and two families: the Acytosteliaceae with one genus, *Acytostelium*, and the Dictyosteliaceae with two genera, *Dictyostelium* and *Polysphondylium* (Olive, 1975; Raper, 1984). Recently, Romeralo *et al*. (2012) reported the number of dictyostelid species to be more than 150 species which belongs to eight major groups based on phylogenetic analyses of both morphological and molecular data. Dictyostelids are also characterized mainly by their fascinating life cycle. Independent myxamoebae consume bacteria by phagocytosis during their vegetative feeding phase. When their food source gets depleted, the myxamoebae enter the second stage of their life cycle wherein free-living myxamoebae come together to form communal aggregates of cells. These

multicellular aggregates differentiate into mature fruiting bodies (Swanson *et al*., 1999). The behavior of cellular slime molds has been shown to be influenced by several cues, namely, light, temperature and gas gradients (Bonner & Lamont, 2005). They tend to feed below the soil and only migrate upward to form fruiting bodies at the surface where the dispersal of their spores via animal transporters is maximized. At the surface, their spores adhere to invertebrates and other transporters that eventually bring them to new sources of bacteria/food. Migrating cells that eventually form the spores are known to be phototactic, which helps guide them move towards the surface during daytime. They are also responsive to thermal gradients. During the day, migrating cells move from below the soil towards the warmth of the surface. They continue to move up even at night when they become negatively thermotactic and move towards the now cooler surface. These cells are also sensitive to gas gradients. It is known that slugs and fruiting bodies emit ammonia gas, which repels developing fruiting bodies from one another. In an experiment conducted by Bonner & Lamont in 2005, they were able to show how fruiting bodies move towards areas with lower concentrations of ammonia. This was demonstrated using a piece of charcoal placed on the surface of the agar plate where the cellular slime molds were grown. The charcoal absorbs ammonia and thus creates an area of lower concentration. Slugs have also been shown to move towards areas with greater oxygen concentration such as the surface layer of the soil in nature. This behavior is important because it explains why clones of cellular slime molds cultivated on agar plates grow away from each other and are not clumped together in indistinguishable colonies or masses like bacterial cultures.

Cellular slime molds are commonly cultivated in the lab as two-membered cultures. They are typically grown on Hay Infusion Agar (HIA) in the presence of a selected bacterium, typically *E. coli*, which acts as their food source. Other bacteria, yeasts, or a combination of different bacteria can be substituted when isolating slime molds with different host requirements such as certain species of *Protostelium* (Cavender & Raper, 1965a). *E. coli*, however, has always remained the food bacterium of choice in any ecological study of dictyostelids since its recommendation by Cavender & Raper (1965a). Cavender and Raper's isolation method for the isolation of Acrasieae (1965a) is considered by most researchers to be the standard method for cellular slime mold isolation and cultivation. In this study, the feeding preferences of the dictyostelid species isolated from Lubang Island, Occidental Mindoro in the Philippines was assessed to determine how well these species would grow when provided with different species of bacteria and yeasts. The present study would provide information that could help optimize techniques used for the isolation and cultivation of cellular slime mold species for future research. Understanding their feeding preference also gives insights into their physiology as well as their potential applications in medical biotechnology.

In this research study, dictyostelids were isolated from soil samples collected from the main island of Lubang in Occidental Mindoro, Philippines following the protocol of Cavender and Raper (1965a). Identification of the isolated dictyostelids was done by comparing their morphologies with those of published literature. The species of dictyostelids used in the study were as follows: *Dictyostelium aureo-stipes*, *D. discoideum*, *D. laterosorum*, *D. mucoroides*, *D. purpureum*, *Dictyostelium* sp. L08-09, *Polysphondylium pallidum*, and *P. violaceum,* and were described in Yulo & dela Cruz (2011). These dictyostelids were maintained by transferring the terminal sorus of a mature dictyostelid fruiting body onto a freshly prepared HIA plate containing a streak of *E. coli*.

To assess the feeding preferences of the isolated dictyostelids, three species each of gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), gram-positive bacteria (*Micrococcus luteus, Bacillus subtilis*, and *Staphylococcus aureus*), and yeasts (*Saccharomyces cerevisieae, Candida famata*, and *Rhodotorula* sp.) served as food source. The bacterial strains were grown and maintained on Nutrient Agar (NA) slants while the yeasts were grown on Potato Dextrose Agar (PDA) slants, all were incubated at room temperature for 24 hours. The feeding preference assay was then conducted on HIA plates containing streaks of the selected bacteria/yeasts. To prepare the plate, 10 µl of the food bacterium or yeast (cell concentration equivalent to 0.5 Mcfarland) was transferred to predetermined points on the HIA agar surface (Fig. 1). An inoculating needle was then modified to make a 5 mm wide and 50 mm long streak of the food microbes that the dictyostelid isolate could consume. Each plate could hold as many as three streaks of the food bacterium/yeast. Then, a single terminal sorus of a dictyostelid isolate was transferred to the end of the streak. The inoculated culture plates were then incubated at room temperature under diffuse light for up to 4 days. The feeding rate expressed in µm/hour was then computed by measuring the distance reached by the "feeding front" from the point of inoculation divided by the total number of hours of incubation. The feeding front is composed of actively reproducing amoebae that crawl on the agar surface following the path of the streak. Behind it lays aggregations and fruiting bodies that form on agar that is essentially denuded of food organism (Horn, 1971). The feeding rates of all isolated dictyostelids on each of the food bacteria or yeasts were presented as graph. To determine the significant difference between the different feeding rates of the isolated dictyostelids, student t-test was computed using XLStat.

Among the three groups of test microorganisms used in the feeding preference assay, the gram-negative bacteria were found to promote the growth of cellular slime molds best (Fig. 2A). *E. coli* remained the preferred food bacterium followed by *P. aeruginosa* and *K. pneumoniae.* Perhaps, the presence of capsule in *K. pneumoniae* affected the abilities of the isolated dictyostelids to consume this bacterium. However, all of the eight isolated species of

dictyostelids fed on these gram-negative bacteria, suggesting preference for this bacterial group. On the other hand, not all dictyostelids fed on gram-positive bacteria (Fig. 2B). Each of the isolated dictyostelids fed on either one or two of the test gram-positive bacteria. Six species utilized *M. luteus*, four species fed on *B. subtilis*, while only three species utilized *S. aureus* as food bacterium. Only *P. pallidum* fed on all of the three gram-positive bacteria, though its feeding rates were lower than most of the isolated dictyostelids. Also, the isolated dictyostelids did not appear to have any preferences for any of the gram-positive bacteria. Furthermore, the feeding rate remained faster with gram-negative bacteria than with these gram-positive bacteria (Fig. 2, Table 1), supporting the use of *E. coli* as the food bacterium for the isolation of these organisms.

It is also interesting to note that only four species grew when yeasts were used as food source (Fig. 3). Minimal growth was observed in the presence of these yeast species: *Candida famata, Rhodotorula* sp., and *Saccharomyces cerevisiae*. From our study, it can be inferred that dictyostelids prefer bacteria as food over yeasts because they have a relatively simpler structure that is easier to digest. The larger size of the yeast cells may also contribute to their preferences for bacteria. Feeding preference could also be affected by attractants produced by different bacterial species. This was demonstrated by Konijn (1969) in a study he conducted about the effect of bacteria on the chemotaxis of the cellular slime molds, although he likewise concentrated on the effects of *E. coli*.

In this study, the growth rate of the dictyostelids was measured at only two time intervals, initially at the time the bacterial streak was inoculated with the terminal sorus of the dictyostelid and after 2-4 days of incubation. It was not necessary to perform multiple measurements over a prolonged period of time because as Horn (1971) observed, the feeding front of cellular slime molds have a virtually constant rate of movement. It is noteworthy to mention that this study is among the few studies made about the feeding behavior of cellular slime molds. Other published studies about dictyostelid feeding behavior have only been those by Hohl & Raper (1963), Konjin (1969), and Horn (1971). Hohl & Raper (1963) looked at how cellular slime molds behave when grown on dead versus living bacteria. Konjin (1969) looked at the effect of bacteria on the chemotaxis of dictyostelids while Horn (1971) looked at food competition among cellular slime molds. This study differs from the investigation done by Horn by using (1) pre-determined, identified food organisms, and (2) by looking at the individual growth rate of dictyostelids on all test bacterial and yeast species. Horn's study was the reverse of these points in the sense that he used (1) only *E. coli* together with unidentified strains of bacteria, and (2) only determined which dictyostelid species among a pool of species would be the most successful in terms of competing for food utilization.

In summary, of the three groups of test organisms (gram-negative bacteria, gram-positive bacteria, and yeasts) used, gram-negative bacteria, particularly *E.*

coli, produced the most prolific growth of cellular slime molds. All eight isolates were able to grow on the three species of gram-negative bacteria. The isolated dictyostelids from Lubang Island, Occidental Mindoro were not "picky" with their choice of gram-negative bacteria. Likewise, all eight species of dictyostelid isolates were able to grow on gram-positive bacteria but only on selected species. Minimal growth was observed when yeasts were used as the food source of the cellular slime mold isolates. In fact, only half of the dictyostelid isolates grew when yeasts were used, and even so, growth was abysmal. These findings only further support the use of *E. coli* in ecological studies of dictyostelids.

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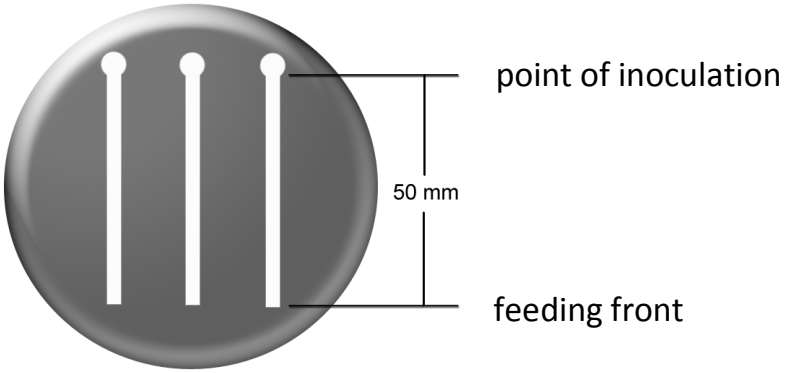

Figure 1. HIA plate used for the feeding preference assay. Each plate contains three streaks of the food bacterium or yeast.

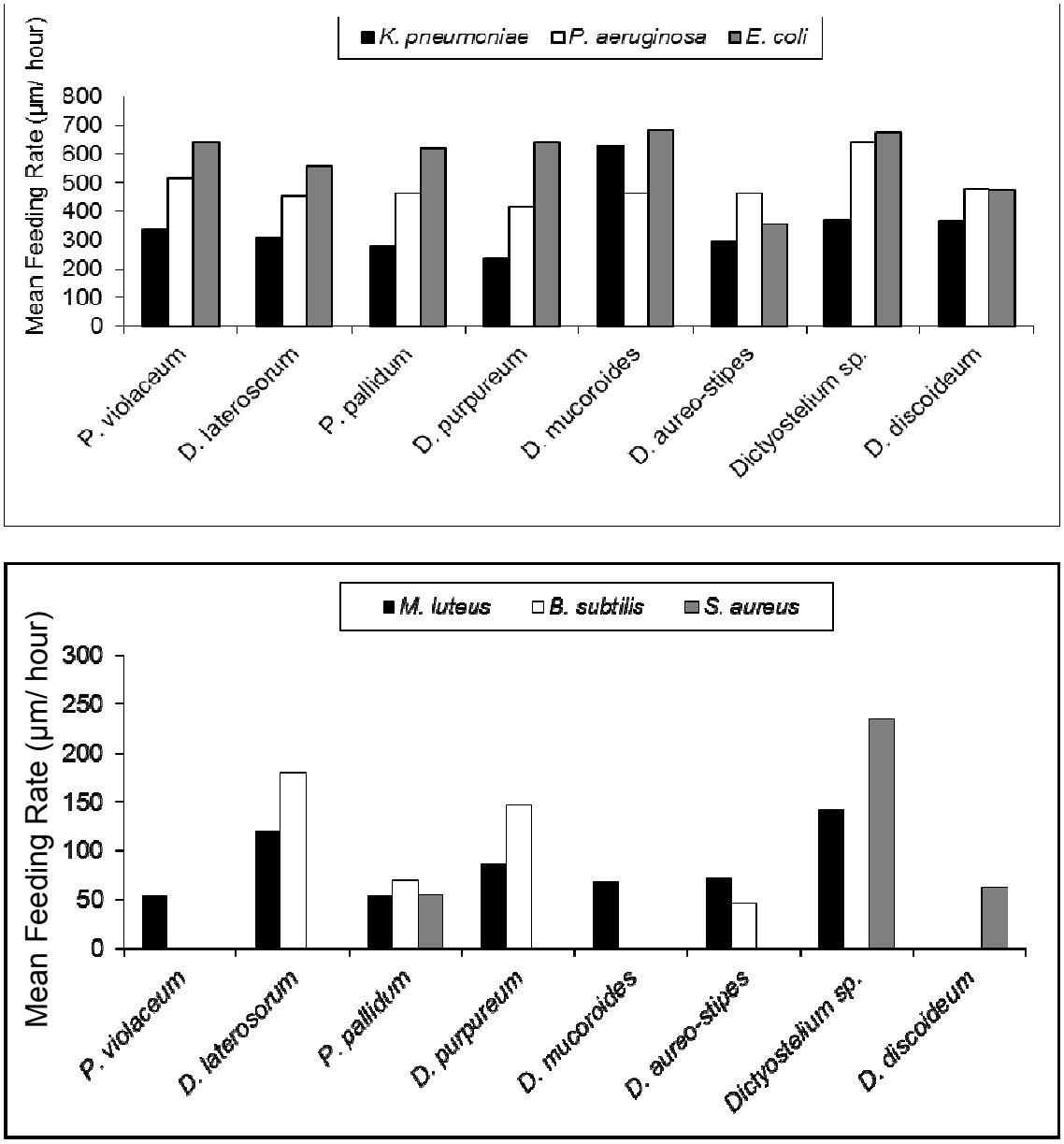

Figure 2. Feeding rates of the isolated cellular slime molds on: (A) gram-negative bacteria: *E. coli, K. pneumoniae*, P. aeruginosa, and (B) gram-positive bacteria: *B. subtilis, M. luteus, S. aureus*.

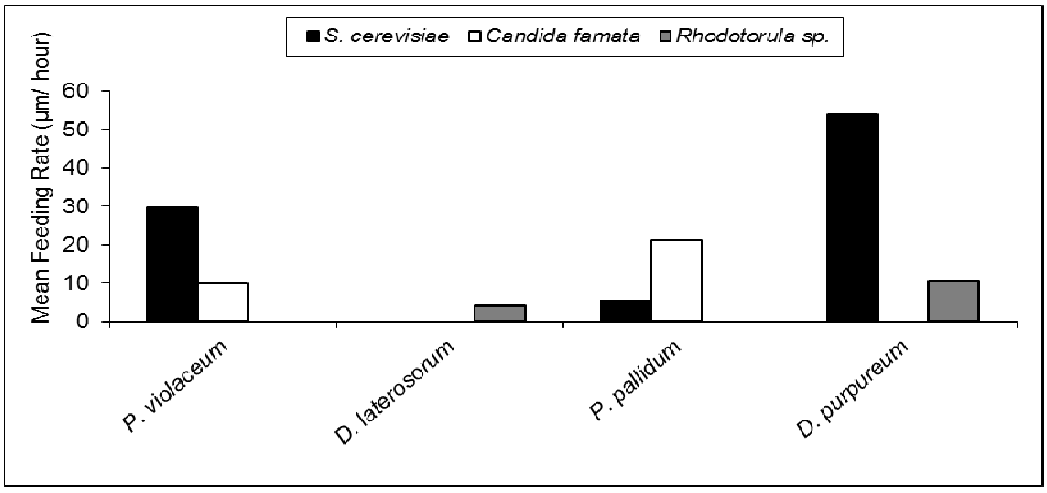

Figure 3. Feeding rates of the isolated cellular slime molds on the following yeasts: *Candida famata, Rhodotorula* sp., and *Saccharomyces cerevisiae*.

Table 1. Mean feeding rate of the eight dictyostelids isolated from Lubang Island, Occidental Mindoro.

	Taxon	**Number of dictyostelid species that consumed the food organisms**	**Mean Feeding Rate (in µm/hr) + SD**
Gram-negative Bacteria	*E. coli*	8	555 ± 113
	K. pneumoniae	8	326 ± 121
	P. aeruginosa	8	455 ± 67
Gram-positive Bacteria	*B. subtilis*	4	111 ± 63
	M. luteus	7	85 ± 34
	S. aureus	3	117 ± 101
Yeasts	*C. famata*	2	16 ± 8
	Rhodotorula sp.	2	7 ± 5
	S. cerevisiae	3	30 ± 24

[a] Student T-test showed no significant differences between the feeding rates of the three species of yeasts and gram-positive bacteria, and between *K. pneumoniae* and *P. aeruginosa*.