Slime fungi: the brainless super microorganism

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Myxomycetes, commonly known as plasmodial slime molds, are unique organisms which take an intermediate position between plant and animal kingdoms. They can form a jelly-like plasmodia which feed on bacteria and are able to move by a synchronized perpendicular flow of their protoplasm. Slime molds can be classified into three groups: cellular, plasmodial, and net slime molds. These interesting organisms are novel sources of several bioactive secondary metabolites with anticancer, antioxidant, and antimicrobial activities. In this review, the ecology, occurrence, and secondary metabolites secreted by these unique organisms are highlighted.

Keywords:
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Introduction

Myxomycetes (plasmodial slime molds) are a group of fungus-like organisms usually present and sometimes abundant in terrestrial ecosystems. The myxomycete life cycle involves two very different trophic (feeding) stages, one consisting of the uninucleate amoebae, with or without flagella, and the other consisting of a distinctive multinucleate structure, the plasmodium. Myxomycetes are unique organisms which take an intermediate position between the plant and animal kingdoms. In a certain stage of their life cycle, they form jelly-like plasmodia which feed on bacteria and are able to move by a synchronized perpendicular flow of their protoplasm [1–4].

Myxomycetes plasmodia (slime molds)

Myxomycetes plasmodia (slime molds) are heterotrophic organisms that were once regarded as fungi but later on they were classified with the Protista. Based on the analysis of nucleic acid sequences, slime molds have been classified as eukaryotes [1]. There are more than 900 species of slime molds all over the world, and they gained their name 'slime' due to their gelatinous appearance. The size of the slime molds may range from few centimeters to several square meters [2]. Phenotypically, slime molds are similar to both the protozoa since the slime molds move like amoeba (gliding motility) and the fungi as they produce spores. However, phylogenetically, slime molds are closer to the amoeboid protozoa than to the fungi [3].

There are three groups of slime molds, including, the cellular slime molds that are composed of single amoeboid cells during their vegetative stage or the plasmodial slime molds (vegetative acellular slime molds) that are made up of plasmodia, amorphic masses of protoplasm. The third group is known as net slime molds (*Labyrinthula*), which are characterized by the formation of an ectoplasmic net [3].

Ecology of *Myxomycetes plasmodia*

M. plasmodia typically occur in cool, moist, shady places such as within the crevices of decaying wood, beneath the partially decayed bark of logs and stumps or other organic matter retaining abundant moisture, and in leaf litter on the forest floor. Under favorable conditions, the plasmodium gives rise to one or more fruiting bodies containing spores. The spores of myxomycetes are for most species apparently wind dispersed and they complete their life cycle by germinating to produce the uninucleate amoeboflagellate cells. Some environmental factors including stress and nutrients can lead to cell accumulation and differentiation into fruiting bodies that in turn lead to spore production. This is followed by spore germination that become vegetative amoeboid cells. They are found mainly in moist environments where they can grow on decaying organic matter. Slime molds feed by engulfing bacteria and various microorganisms through a process known as phagocytosis. Light is also necessary to induce sporulation in several species [3].

Plasmodial slime molds (myxomycetes or true slime molds)

The plasmodial slime molds are phagotrophic eukaryotes; these enormous single cells with thousands of nuclei are mostly found in terrestrial ecosystems. When individual flagellated cells swarm together and get fused myxomycetes are formed. The obtained large bag is formed mainly of cytoplasm with many diploid nuclei. The resultant giant cells are considered very useful in studying the streaming of the cytoplasm that includes the movement of other cell contents since this process can be visualized by the mean of relatively low magnification. Another factor that makes it easier to manipulate these slime molds is its relatively large size [4] (Fig. 1).

Since the middle of the 17th century, plasmodial slime molds have been recognized from their fruiting bodies when the German mycologist Thomas Panckow described a member of this group (*Lycogala epidendrum*) [1]. This was followed by the description of *Stemonitis* species by Domke, [4,5], whereas Waggoner and Poinar [6] described a myxomycete plasmodium fossil in amber from Eocene-Oligocene that exits in the Dominican Republic. Moreover, Dörfelt *et al.* [7] described *Arcyria* spp. Nearly, 875 species of plasmodial slime molds have been reported [8], and have been placed in six various taxonomic orders (Echinosteliales, Ceratiomyxales, Physarales, Stemonitales, Liceales, and Trichiales). However, Ceratiomyxales members were found to be different from other order members, and have been reassigned as protostelids [9–11]. While the myxomycetes evolutionary are still debated, these organisms formed a well-defined group.

Figure 1

Plasmodial slime molds-plasmodium (photographs taken by Clayton, Michael. Locality: University of Wisconsin–Madison Botany Department, hosted by https://search.library.wisc.edu).

Factors affecting the occurrence of plasmodial slime mold (myxomycetes or true slime molds)

There are many factors that greatly affect the occurrence of myxomycetes in nature [12], among which are temperature and moisture. Moreover, the occurrence of these species tends to increase with increasing the biomass and the diversity of the vascular plants, which provide various resources required to support the growth of bacteria and various microorganisms consumed by the myxomycete [13,14]. Myxomycetes gain their common name (slime molds) due to the production of a pronounced amount of slimy materials during a stage in the life cycle known as the multinucleate trophic stage (plasmodium). Some of this slime could be left behind as the plasmodium migrates over a particular substrate's surface and this is known as 'slime track.'. There are two different trophic stages in the myxomycete life cycle, the first stage consists of uninucleate amoebae and the second consists of distinctive multinucleate structure, which is the plasmodium which in turn converts into fruiting bodies under suitable conditions [15]. These stages in the myxomycete life cycle make them unusual groups of microorganisms that exhibit both fungi and protozoan characteristics.

Plasmodial slime mold (myxomycetes or true slime mold) secondary metabolites

The secondary metabolism of myxomycetes remained virtually unknown. Several bioactive secondary metabolites have been isolated from myxomycetes [16]. Some anticancer compounds have been isolated from myxomycetes, such as cyclic phosphatidic acid, which is a novel bioactive lipid that inhibits cancer cell invasion as well as the metastasis [17]. Moreover, some antimicrobial compounds such as a bahiensol (new glycerolipid) as well as stigmasterol and fatty acids were obtained from some plasmodial extracts [18]. Interestingly, extracellular polysaccharides (EPS) isolated from *Physarella oblonga* showed in-vitro antioxidant activities with an EPS concentration range of 0–6.0 mg/ml. This activity was determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay where ascorbic acid was used as a positive control. The results showed that EPS from *Physarella oblonga* showed maximum DPPH scavenging activity which was 80.41% at a concentration of 6 mg/ml EPS, whereas that of

ascorbic acid (positive control) was 99.56% [19]. Also, EPS in-vitro cytotoxicity toward cancer cell lines was evaluated using the sulforhodamine B assay. Cytotoxicity of EPSs ranging from 0.25 to 1.5 mg/ml were evaluated after an incubation of 48 h. The results indicated that EPS exhibits inhibitory activities against MCF-7 (human breast adenocarcinoma cell line) and HepG2. IC_{50} (the half-inhibitory concentrations) of EPS sample HepG2 and MCF-7 were found to be 1.11 and 1.22 mg/ml, respectively [19].

Cellular slime mold

The cellular slime molds belong to the order Acrasiales which is within the phylum Myxomycophyta. Although various genera and several species have been reported; the major investigations have been focused on the species *Dictyostelium discoideum*. This species has the ability to construct fruiting bodies under variable environmental conditions; additionally, the morphogenetic sequence of this species has been well defined, (Fig. 2). Two more related species, *Dictyostelium purpureum and Dictyostelium mucoroides* have gained importance for current investigations. *Polysphondylium pallidum*, another genus member, has gained great importance as it has been the first cellular slime mold cultivated on defined media [20,21]. However, one of its drawbacks is that it constructs its fruiting bodies more slowly than *D. discoideum* and it is even more fastidiously. Hence, *D. discoideum* has been reported to be the species of choice for conducting current experiments for the study of cell and developmental biology [22]. The numbers of cellular slime molds species decrease with increasing the elevation and the latitude. Many of cellular slime mold species appear to be highest in the American tropics. Also, many different species have been found around the Mayan ruins at Tikal in Guatemala.

Figure 2

Cellular slime mold (photographs taken by Uno Eliasson. Locality: University of Gothenburg, Department of Biological and Environmental Sciences, hosted by https://unoeliasson.commyxomycetes.html).

First, the vegetative cells of *D. discoideum* grow as single amoebae and they feed on bacteria, but once get starved, they gather and develop a slug shaped, multicellular aggregate. In turn, the formed aggregate will differentiate into two cell types, prespore and prestalk cells. The two cell types are considered precursors of spores and stalk cells, respectively. Finally, the aggregate develops a fruiting body formed of spores and a multicellular stalk. Unlike other spore-producing organisms, spores produced by the cellular slime molds cannot be carried over long distances by wind; however, their dispersal depends mainly on their transport accidentally after being carried on some animals body surface or within their digestive tract [23].

Cellular slime mold secondary metabolites

Several important bioactive compounds have been isolated form cellular slime molds, including differentiation-inducing factors (DIFs) [24], cAMP4 [25] and discadenine [26], and have been observed as development-regulating substances. DIF-1 was reported to inhibit the proliferation and induce differentiation in mammalian leukemia cell lines [27]. DIF-1 may also induce vascular smooth muscle cell differentiation *in vitro*, which could lead to great achievements in the treatment of various vascular diseases [28]. Additionally, a chlorine-substituted aromatic compound known as AB0022A with antibacterial activities has been isolated from cellular slime molds [29]. A new aromatic amide known as brefelamide was also isolated from the methanolic extracts of *Dictyostelium brefeldianum* and *Dictyostelium giganteum* fruiting bodies and, interestingly, this compound has been reported to inhibit the proliferation of 1321N1 human astrocytoma cells [23].

Net slime molds (Labyrinthulidae)

Labyrinthulidae or net slime molds are a small group of aquatic or terrestrial saprophytic protozoans. Being non-photosynthetic (like fungi) and producing motile zoospore (like algae), they were transferred from the kingdom 'Fungi' to the kingdom 'Protista' [30]. They have a unique plasmodial stage which is made from structurally distinct trackways with spindle-shaped cells gliding across them [31]. These trackways form a network by interconnecting the aggregates of cells found over the substratum, and hence gain their earlier name 'net slime molds.' This plasmodial network is very complicated morphologically and

plays an important feeding role [32]. In 1930s, *Labyrinthula* gained its importance as scientists discovered that it was the main cause of 'wasting disease' of seagrass spread on the European and North American coasts. Since then, several pathogenic species that live in marine water have been identified [33]. *Labyrinthula* is commonly found to live on seaweed [34] and diatoms [35], and sometimes they are found on marine angiosperms.

The studies confirmed the existence of both pathogenic and nonpathogenic strains of *Labyrinthula* [36]. Moreover, *Labyrinthula* spp. was found to be a terrestrial plant pathogen, and hence it has shown to be the main cause for the 'rapid blight' disease that spread among turf grasses in Arizona, where irrigation water is characterized by its high salinity [37]. Although *Labyrinthula* life cycle is not completely understood, production of flagellated zoospores was reported and their development was followed at the ultrastructural level [38]. The trackways were proven to be formed of cytoplasm which is surrounded by a plasma membrane [39]. In addition, specialized junctions between the cytoplasmic network and the spindle cells have been found. These complicated ultrastructures are named bothrosomes as they exhibit pit-like depressions [40] with dimensions of 0.3 mm deep and 0.3 mm across; about 10–20 spindle cells are estimated to be found over their surfaces [40].

Isolation of slime molds

The isolation and cultivation of slime molds have been studied in many literatures especially molds such as the cellular slime mold *D. discoideum*, which represents one of the excellent model organisms for the study of cell, feeding habits, and developmental biology due to its simple life cycle and ease of use [41]. Generally, slime molds can be isolated from different environments such as regular soil, forest soil, dungs of different animals, decaying mushrooms, decomposing grass, rotting vegetables, compost, rotting wood, or musty hay [42–45].

Many methods have been described to isolate slime molds, such as placing small quantity of the sample on the surface of a non-nutrient agar plate and incubate plates at 20–24°C to permit development of slime molds upon or adjacent to the material. Once fruiting structures have developed, spores are removed from sorocarps believed to be free of contaminating organisms, and was purified by planting them in streak colonies of a selected and previously established bacterial associate growing on some nutrient-poor agar medium. *Escherichia coli* is one of the suitable bacterial associates for such mission among other species [43].To obtain the slime mold in pure-mixed culture with the chosen bacterial associate, the bacterial colonies were cross-streaked on the plate. The spores of the slime mold are then planted at the intersection spaces of the two streaks. When the slime mold grows, a small inoculum of vegetative myxamoebae or spores is removed and replanted in fresh cultures of the selected bacterial associate on similar nutrient-poor agar medium. After that, the process should be repeated and myxamoebae or spores should again be removed and planted in a new plate with bacterial colonies. In some cases, this process needs to be repeated further to obtain a pure-mixed culture. This method is conditioned by many factors as growing the slime mold, when first transplanted, on some rapidly spreading species of bacteria. It should be mentioned that the cleaner the spore head is at the time of transplantation, the less the difficulty that will be encountered in placing the slime mold in a pure-mixed culture with a particular bacterial associate [42,43].

The second method of isolation is conducted through mixing small quantities of sample with an appropriate amount of sterile water, then streaking the resulting suspension on strength hay agar, and incubating plates at 20–24°C. After 3 days of incubation, plates are examined for the appearance of the wheel-like aggregating pseudoplasmodia which are characteristic of the members of this group during the initial stages of fructification. As these structures are spotted, the fungus which overlie the aggregating myxamoebae should be removed and transplanted directly to the intersection of crossed streaks of a desired bacterial associate growing upon a suitable nutrient-poor medium. Another method can be performed after spotting the wheel-like aggregating pseudoplasmodium position, which is by marking the pseudoplasmodium in the original streak plate, and by delaying the transplantation process until the fruiting structure becomes mature spores. Then, it can be removed from the elevated sorus, or spore mass, and is transplanted to a plate containing previously growing bacterial colonies. In either case further purification is the necessary following step.

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Conflicts of interest

There are no conflicts of interest.

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