



The Ancestors of Cryptococci Appeared on Earth 600 Million Years Ago

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Abstract

The purpose of the research is to study the early forms of life in the Neoproterozoic. To achieve this goal, the author has conducted the search for microfossils in stromatolites and has systematically identified the detected forms. In Eastern Siberia, 600-million-year-old Neoproterozoic rocks host columnar stromatolites. They were formed in the sublittoral areas of the warm sea and consisted almost entirely of mineralized (silicified) remains of yeast fungi that, during their life cycle, parasitized the green algae thallus. The combined presence of fungi and algae was established in stromatolite samples using a scanning electron microscope (SEM). In the same stromatolite sample, the world's first well-preserved mineralized (silicified) early forms of cryptococci were discovered. They are represented primarily by round, individual budding cells that consist of central and peripheral parts. The found microorganisms were identified into a new genus and type species. The latter evolved as yeast fungi and had characteristics similar to the modern *Cryptococcus neoformans*, from which it differed in a smaller size. It appears that the early ancestors of cryptococci, which invoke Cryptococcosis, occurred on Earth at circa 600 Ma. For such a long period (part of the Neoproterozoic and the entire Phanerozoic) of their presence on Earth, the environment conditions have repeatedly changed from aquatic to terrestrial. This may have led to the fact that cryptococci, unlike the Neoproterozoic ancestors, became more advanced in physiological terms (evolutionarily advanced), and could adapt to various environments and affect human lungs, skin, limbs, etc.

Subject Areas

Microbiology

Keywords

Ancestors of Cryptococci, Cryptococcosis, Cells, Yeast Fungi, Algae, Stromatolites, Neoproterozoic, Eastern Siberia

1. Introduction

Research on the early stages of the development of life on Earth is of great scientific significance. Many taxa of bacteria, cyanobacteria, and algae have been described from the rock layers formed on the continents during the Precambrian [1]. The least amount of data was available on fungi, for which a synthesis of data was recently made by A. Tomescu *et al.* [2]. According to the suggestions of many scientists and data of poorly preserved fossils summarized in the press [3], fungi appeared in the Precambrian (about 541 Ma).

During the late Ediacaran (about 600 Ma), large territories of Eastern Siberia (Siberian platform) contained a huge epicontinental marine basin in which many different microorganisms existed. For this reason, the east of the Siberian platform represents the most suitable territory for research on the diversity of microorganisms in the Ediacaran. A systematic study of the Ediacaran microorganisms of the area was started by the author in 1964. Cyanobacteria, bacteria, algae, and fungi are described in various (calcified, silicified, and organic-walled) types of preservation [4].

Microfossils that are slightly similar to the yeast spores (in globular shape; size (4.7 - 20 μm); and dark spots inside (up to 1 μm) that represent degraded remains of cell contents) described in the manuscript were previously found (in transparent sections) in several parts of the world. They were not considered as possible spores of yeast fungi and were mistaken for the remains of cyanobacteria. Among these microfossils, several cell-like bodies of the Yudoma formation in the Ediacaran Eastern Siberia [5] [6] were mostly similar in shape, size and presence of a dark spot to the spores of yeast fungi described in this manuscript. This can be considered as one of the proofs that the Byuk early forms of cryptococci are of Ediacaran age, not the late ones.

2. Materials and Methods

The Byuk formation (geological formation, body, 600 Ma rock layers) of the Upper Ediacaran is confined to the Berezovsky trough in the southeast of the Siberian platform (southwest of Yakutia). The site of the collection of material from this formation was provided earlier in another article [7]. In this formation, columnar silicified stromatolites (stone formations resembling coral reefs in shape) are widespread. They were formed in the sublittoral of the theperitidal (flooded and adjacent unflooded and partially flooded areas) zones of the warm sea. Stromatolites almost entirely consist of mineralized (silicified) remains of microorganisms. Thus, microfossils, *i.e.*, the remains of microorganisms, described further below in the manuscript are certainly *in situ*. This is particularly seen in the photographs provided (**Figures 1-3**, parts of the fungi in the rock).

According to Fadeev A. S. [8], cryptococci are single budding yeast cells. Fungi are characterized by the round, rarely oval, yeast cells of 6 - 13 μm in size, occasionally up to 20 μm , surrounded by a capsule that can reach 5 - 7 μm in size and sometimes exceeds the diameter of the vegetative cell. The natural source of

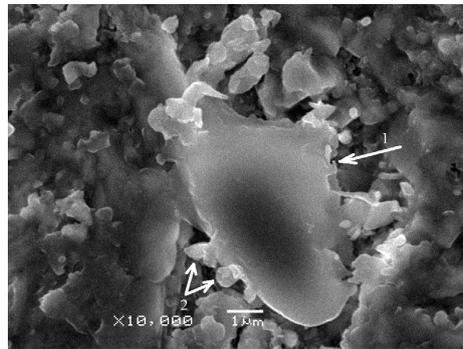


Figure 1. Single budding yeast cell. 1—cell; 2—buds.

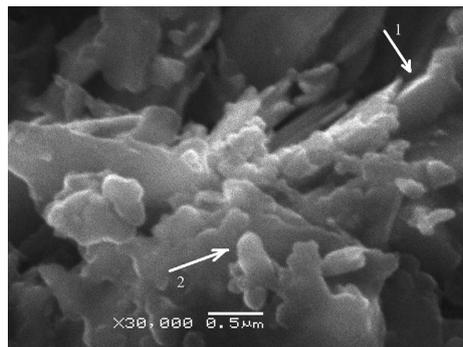


Figure 2. Yeast parasitizing the thallus of the lamellar green algae. 1—a fragment of a lamellar green algae; 2—yeast cells with multiple outgrowths.

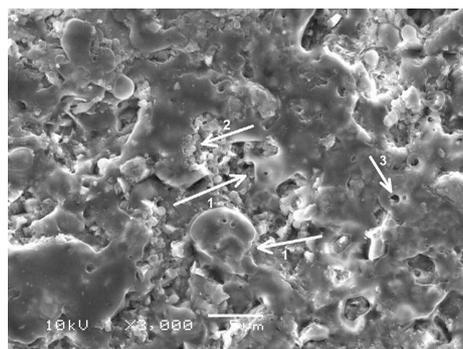


Figure 3. Yeast respiration. 1—cells grow by forming outgrowths; 2—yeast reproduces by budding; 3—pores formed by cell respiration; occasionally, the spores experience the release of organic matter.

Cryptokokkus neoformans var. *neoformans* is the soils containing pigeon droppings, and less frequently rotting vegetables, fruits, and plants. Cryptococci are unpretentious and can grow quite easily in normal environment conditions. They are characterized by a sufficiently high stability in the environment and are sensitive to the temperature changes. Infection of a person with cryptococcosis is a serious pathology. The disease is widespread; however, there are regions with higher incidence of pathology, e.g., Japan, North America, and European countries. *Cryptococcus neoformans* (Figure 4) is a major cause of central nervous system lesions in patients with immunodeficiency.

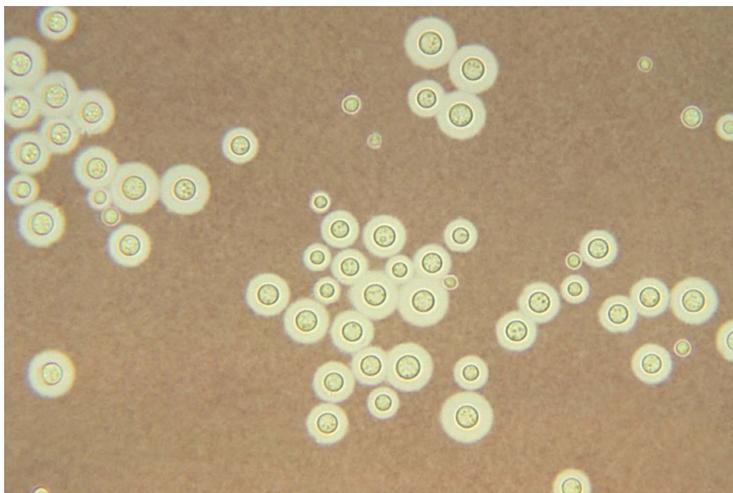


Figure 4. Cryptococci *Cryptococcus neoformans* [13].

The microscopic organic remains of the Byuk formation were investigated by scanning electron microscopy (SEM) in several vertically oriented thin sections acquired from a sample of silicified columnar stromatolite containing fungi *Surinia implicate* and *Sakhi solomonovi* [7] [9]. The sections were ground using fine powder, washed with distilled water, dried, and coated with gold under sterile conditions. This method is applied by many researchers. The Jeol JSM-6480LV SEM was used under a high vacuum and an accelerating voltage of 10 kV, diaphragm 2, signal SEI, and a working distance 10 mm. Microfossils were studied using the traditional comparative morphological method. Their biological interpretation and systematic identification were conducted using analogy approach, the most common and effective method in scientific research [10].

3. Discussion and Results

The cells of the abovementioned microorganisms are spherical and irregular in shape, demonstrate variety of sizes (in diameter 10 μm or more), and are often present in individuals. Respiration of cells was observed (Figure 3). The microorganisms parasitized algae thalli and lived in an organic-rich algal environment in which they reproduced by sporogenesis, division and budding. Besides separation, budding cells are capable of development into chains (conidiospores). Characterized microorganisms are fungi. They were capable of intensive asexual reproduction by budding (Figure 1 and Figure 4 and Figure 5) and division (Figure 4 and Figure 5, arrow 8). Like recent cells, yeast cells (a yeast cell consists of a single body) of the described fossils [11] were developed after the formation of outgrowths (Figure 2). Most cells have pores of varying diameters, that are formed as a result of their respiration (Figure 3), typical for yeast. Occasionally, a small amount of organic matter is released in the process (Figure 3). Based on these characteristics, the fungi were classified as single budding (Figure 1) saccharomyces yeast (*Saccharomycotina* subdivision of the *Saccharomycetes* class).

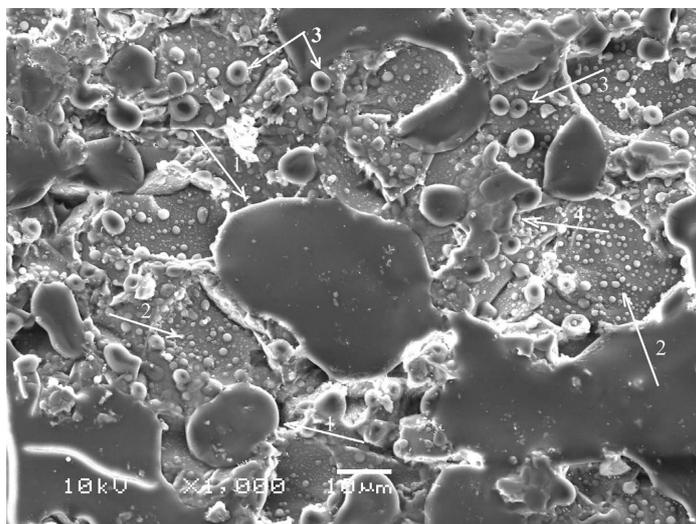


Figure 5. Numerous mother and daughter yeast cells and their nanoscale spores; through the process of development, some spores evolved into early forms of cryptococci: 1—mother cells of the yeast fungi; 2—yeast spores; 3—early forms of cryptococci; 4—aggregates of multiple cells.

In the same stromatolites, a massive formation of globular microfossils has been established (**Figures 5-7**) alongside the cells and buds. They were biologically classified as the yeast fungal spores [12] that function as a means of reproduction by sporogenesis (**Figure 6**, arrow 4). The size of the spores ranges from nanoscale (**Figure 5** and **Figure 6**) to larger ones (diameter 1.2 - 2.0 μm). In the process of development, they transform into cells, whereas many spores resemble cryptococci (**Figure 4**).

Many spores are observed. They are located separately and are less frequently observed in the form of conidiospores that are located in chains (**Figure 6**, arrow 4, 10). The spores ripened at a different time; therefore, they differ in size. Spores with a diameter of 0.3 - 1.2 μm prevail (**Figure 5**, arrow 2). The spores of the fossil yeast, as well as the recent ones, caused the transfer of the adverse environmental conditions. Their main function, however, is reproduction. They transform into cells (2.5 - 2.8 μm in diameter, rarely more) as a result of the spore growth. The spores consist of central and peripheral parts (**Figures 5-7**). They are like cryptococci in their rounded shape, structure, and size. Presumably, they are early forms of cryptococci *i.e.*, single budding yeast cells (**Figure 5**, arrow 3). In the photographs, their central part appears as a dark spot (**Figure 5** and **Figure 6**). In the fossil material, it was not possible to identify the content of the central part of these spore cells. Similar to cryptococci (**Figure 4**, [13]) one can only assume the presence of organelles in it (mitochondria, vacuoles, granules of replacement nutrients, etc.). The peripheral part of the cells is similar to the cryptococcus capsule. There are spores that have not yet separated from the mother yeast cells; however, they already have characteristics of the early cryptococci (**Figure 6**, arrow 3). When do yeast spores evolve into forms similar to the early cryptococci? There is no clear answer to this question. It can only be

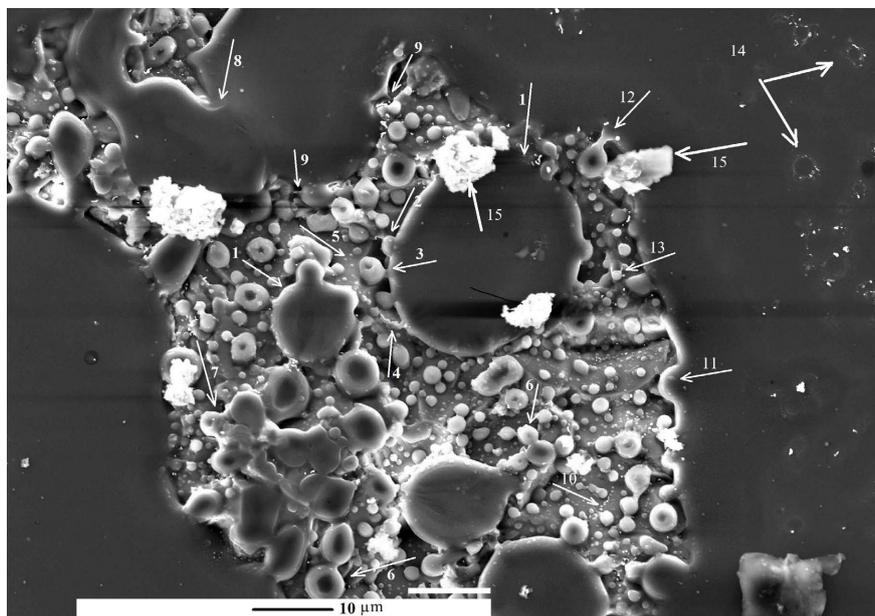


Figure 6. Cells and spores of yeast fungi, formation of the early cryptococci. 1—yeast cells; 2—bud, 3—a bud that has not yet separated from the mother cell, but is already acquiring characteristics of the early cryptococci; 4—process of the nanoscale spores formation; 5—the youngest nanoscale spores located separately; 6—conjoined spores; 7—aggregate of the conjoined cells; 8—daughter cell that has not been separated from the mother cell; 9—spores with very thin and short spikes; 10—nanoscale spores arranged in a chain; 11—nascent buds; 12—bud in the process of separation from the mother cell; 13—spiked spore; 14—traces of buds that have separated from the mother cell; 15—non-organic matter.

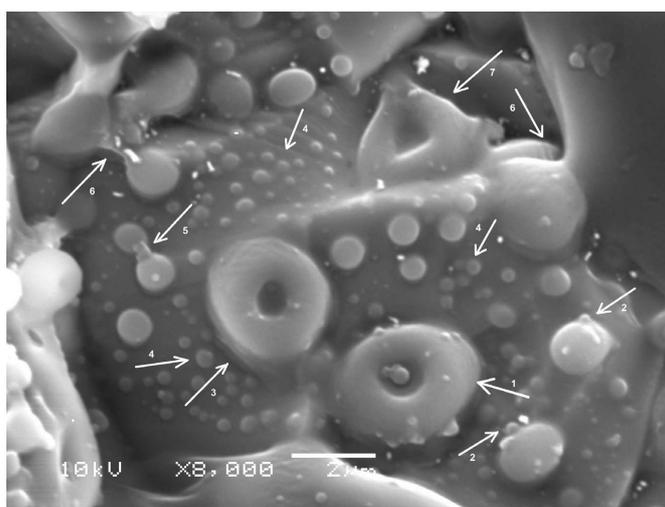


Figure 7. Single budding yeast cells: 1—an early form of cryptococcus with a thick capsule (wall); it has ripened, numerous nanoscale spores are visible on the surface, and there is a spore in the inner part of the cell; 2—reproduction of yeast cells by spores; 3—similar to cryptococci, the surface of the capsule of the early forms of these microorganisms is covered with a barely visible transparent layer that, presumably, was mucosal during lifetime; 4—single spores; 5—conjoined spores; 6—several spores or cells were glued together via mucous layer, forming an aggregate; 7—yeast cell of triangular shape (in cross-section).

suggested that the development of spores as cryptococcus is due the evolution of yeast, presumably stimulated by their environmental conditions.

At a certain stage of development, walls of some yeast cells from the Byuk formation can form a slime layer (Figure 7, arrow 3), resulting in the gluing of individual cells into larger aggregates (Figure 6, arrow 7). This process is like processes observed in the recent yeast, and is known as agglutination, the formation of flocculent yeast.

The exceptional preservation of the Byuk microfossils (Figures 1-3 and Figures 5-7), which are biologically interpreted as the remains of fungi and algae, is presumably related to their microscopic size and to the fact that the wall of fossil fungi may contain chitin, as in those of recent fungi. Another factor of preservation could arise from the occurrence of special taphonomic conditions (burial conditions) triggered by the coincidence in time and place of action of several biotic and abiotic factors. Presumably, there was no interaction of the organic matter of algae and fungi with oxygen-oxidizer; therefore, decomposition of the organic matter of microorganisms did not occur for a period since the oxygen was immediately consumed by rapidly reproducing and growing yeast. It is indicated by an extremely large number of cells and their spores (Figure 5 and Figure 6). Finally, the excellent preservation of microorganisms could also be the result of a rapid silt burial of stromatolites caused by the well-known Late Ediacaran marine transgression in the area.

4. Conclusions

In Eastern Siberia, columnar stromatolites are present in 600-million-year-old Neoproterozoic rocks. They were formed in the sublittoral areas of the warm sea and consisted almost entirely of mineralized (silicified) remains of yeast fungi. In the same stromatolite sample, the world's first well-preserved mineralized (silicified) early forms of cryptococci were discovered. They were formed as a result of the yeast fungi reproduction through sporogenesis. In terms of morphology, they are similar to the recent *Cryptococcus neoformans*, from which they differ in a smaller size. It appears that the early forms of cryptococci, which invoke Cryptococcosis, occurred on Earth at circa 600 Ma. They parasitized the green algae thalli. For such a long period (part of the Neoproterozoic and the entire Phanerozoic) of their presence on Earth, the environment conditions have repeatedly changed from aquatic to terrestrial. This may have led to the fact that cryptococci, unlike their earlier Neoproterozoic forms, became more advanced in physiological terms and could adapt to various environments and thus affecting human health. Due to the lack of data of the microfossil record, it is unknown whether the early forms of cryptococcus existed continuously. It is possible that they appeared repeatedly on Earth, depending on the certain conditions in the environment. The obtained result is an example of one of the first occurrences of microorganisms on Earth that cause various diseases in humans. It indicates the importance of searching and studying the early forms of microorganisms to obtain new knowledge regarding their occurrence patterns, necessary for

prediction of the possible cases.

The studied collection N°87 is kept in the Diamond and Precious Metal Geology Institute, SB RAS (Yakutsk, Russia). This research was funded within the framework of the state assignment.

Conflicts of Interest

The author declares no conflicts of interest.

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