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ELECTRON MICROSCOPY OF *BLASTOCYSTIS HOMINIS* AND OTHER DIAGNOSTIC APPROACHES

By

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Abstract

This work evaluated light microscopy and culture against conventional PCR in diagnosing *Blastocystis hominis* in human stool samples, and were studied by scanning and transmission electron microscope.

Hundred stool samples were acquired from the Parasitology laboratory of the Medical Research Institute in Alexandria. All samples were subjected to light microscopy, culture and conventional PCR to diagnose *B. hominis* in human stool samples; subsequently *B. hominis* obtained from culture media were further studied by scanning and transmission electron microscope.

The results showed *B. hominis* was detected in 52/100 studied stool samples using light microscope, xenic culture showed 65 positive samples, 67 samples were positive by conventional PCR. SEM revealed the outer surface of *B. hominis* and rosette-shaped dividing oocysts while TEM demonstrated internal structures of the organisms.

Keywords: *Blastocystis hominis*, diagnosis, microscopy, culture, PCR

Introduction

Blastocystis hominis (*B. hominis*) is an obligate anaerobic protozoan found in human and animal large intestine, and is the most common eukaryotic organism reported in human fecal samples. Still there is a controversy about considering *B. hominis* as a pathogen or a part of the normal intestinal flora (Tan *et al,* 2002).

The taxonomy of *B. hominis* remains controversial, and not been determined if a single species or multiple species exist in human and animal hosts. *B. hominis* is difficult to classify and may eventually be placed in individual niche within the protozoa, based on the unique diversity of its shapes, sizes, its unique physiology, and some of its reproductive modes. Sharing RNA sequences with other eukaryotic cells confirm that *B. hominis* is a protozoan found in a group by itself (Parija and Jeremiah, 2013).

There are now at least 17 genetically distinct small subunit ribosomal RNA lineages (Parija *et al,* 2013; Tan *et al,* 2008). The host specificity and pathogenic potential of different isolates correlate with sequence variations in the SSU-r RNA. Such species are currently termed "subtypes" (STs).

B. hominis contains one or more nuclei, smooth and rough endoplasmic reticulum, Golgi complex, and mitochondrion-like organelles. A number of morphologically distinct forms of the organism have been described (Stenzel *et al,* 1991).

Forms of *B. hominis* described in stools or in vitro cultures are vacuolar, granular, amoeboid, cyst, multivacuolar, avacuolar forms. Cyst form is the dominant form found in environment (soil and water) acting as a vehicle for transmitting the parasite into the host (Zhang *et al,* 2012).

B. hominis has four modes of division; binary fission, plasmotomy, schizogony and endodyogeny. An additional mode of the reproduction by budding was presented. In the host, division of *B. hominis* is usually by binary fission (Tan *et al,* 2008).

Different diagnostic methods can be used for detection of *B. hominis* such as light microscopy, the *in vitro* cultivation, the

immunodiagnostic assays, molecular tests and TEM & SEM (Garcia, 2016).

The simplest method for *Blastocystis* detection is by using simple smears in normal saline and iodine stain. *Blasto-cystis* spp. is usually identified microsco-pically by the presence of the vacuolar form, which is easily recognized by its large size and characteristic appearance (Suresh and Smith, 2004).

Culture method is the gold standard for detection of *B. hominis*, it proved to be more sensitive in detecting *Blastocystis* spp. but was not commonly used in the diagnostic laboratories. It is time consuming (2-3days) and the results can be biased because the growth of one subtype of *Blastocystis* spp. can overcome other subtypes (Suresh and Smith, 2004).

Molecular diagnosis by polymerase chain reaction (PCR) detection is a useful tool for detecting and analyzing subtypes of *Blastocystis* spp. from stool specimens and is becoming more widely used for the detection of enteric parasites in both humans and animals (Roberts *et al,* 2011).

Transmission Electron Microscopy (TEM) may assist in confirming the dia-gnosis of atypical morphological internal structures of *B. hominis* such as small cyst form, with large lipid or glycogen inclus-ions. Nevertheless, it was generally not re-quested for routine diagnosis of the organism and is of greater significance in research (Zhang *et al,* 2012).

Scanning Electron Microscopy (SEM) revealed external morphological forms of cyst and various surface coat structures (Zhang *et al,* 2012).

This work aimed to evaluate the light microscopy and culture against conven-tional PCR in diagnosing *B. hominis* in human stool samples and by scanning and transmission electron microscope.

Materials and Methods

Fresh stool samples were collected from one hundred participants attending the Pa-rasitology laboratory of the Medical Res-earch Institute, Alexandria.

The stool samples were collected without preservatives in dry, clean, plastic conta-iners with tight fitting lids. Each sample was divided into three parts: First part was submitted to direct saline and iodine wet mounts examined microscopically for *B. hominis* and any associated protozoa cysts, helminthes eggs and larvae (Garcia, 2016). Second part was directly cultured in sterile 7ml screw tubes containing Jones" medium, and incubated at 37°C for 1-3 day (Clark and Diamond 2002). The cultures were examined by light microscopy, samples were subjected to the scanning and transmi-ssion electron microscopy (Zhang *et al,* 2012), and third part was stored at -20°C for DNA extraction to detect *B. hominis* using conventional PCR (Clark, 1997).

Results

The present study enrolled a total of 100 participants randomly selected from those referred for checkup evaluation of infec-tion status. After obtaining an informed consent, a stool sample was collected from each participant to evaluate different diagnostic techniques for diagnosis of *B. hominis*.

Light microscopy: Wet mount examina-tion of stool samples showed that among the 100 participants, 69 displayed an array of parasites. *B. hominis* showed the highest percentage of infection in 52 participants (Fig. 1). Other parasites were: *Dientamoeba fragilis* (19 cases), *Giardia lamblia* (night cases), *Ascaris lumbricoides* (five cases), *Ancylostoma duodenale* (two cases), and one case of each of *Entamoeba histolytica, Hym-enolepis nana*, and *Schistosoma mansoni*. As for multiplicity of parasite infection, some participants were infected with more than one parasite, 46 patients had single parasitic infection, 18 patients had double infections and only five 5 patients had triple infections.

Stool culture in Jones' media to detect *B. hominis* showed that 65/100(65%) cultured samples were positive for *B. hominis* and

only 35 stool samples were negative after 3 days of incubation (Figs. 2, 3).

Conventional PCR amplification of *Blastocystis* SSU rDNA from stool samples of 100 participants was done. Positive controls from samples positive for *B. hominis* and negative controls (DNA nuclease free water) were included. *B. hominis* was detected in 67/100(67%) participants (Fig. 4).

Comparison between the three diag-nostic techniques to detect *B. hominis* in100 stool samples (Fig. 5), showed that conventional PCR gave the highest positive cases (67%), followed by stool culture (65%), while wet mount method gave the lowest ones (52%), but without significant difference in the detection of the *B. hominis* by the three methods. (x=5.5 94 at p=o.o66).

Scanning Electron Microscopy (SEM) examination for *B. hominis* showed different forms that developed in Jones' culture media with the different topographic external morphology both in the shape and size. This parasite could be round, oval, and irregular in shape, and 5-20µm in size. Surface coat was a kind of fibrous substance in variant appearances (rough or smooth) attaching to the cell surfaces of variant *B. hominis* forms (Figs. 6 to 10). Reproduction of *B. hominis* was observed by binary fission and rosette formation was clearly observed. (Figs. 8 to 10).

Transmission Electron Microscopy (TEM) examination for *B. hominis* from the stool cultures showed different forms developed in Jones' culture media by the different ultrastructure morphology.

The vacuolar form was commonly found in fecal samples and culture. It was round or oval in shape, with an average size varying from 5-14µm, which was covered by a surface coat and possessed a characteristic central vacuole with scanty fine granules and surrounded by peripheral rim of the cytoplasm containing a single nucleus, other organelles and smaller vacuoles (Fig. 11).

Granular form present was round or oval in shape and similar to vacuolar form in size and covered by a surface coat. The central body was almost filled with granules of different electron densities and surrounded with peripheral rim of the cytoplasm containing mitochondria-like-organelle, other organelles and smaller vacuoles (Fig. 12).

Discussion

B. hominis is an obligate anaerobic protozoan found in human and animal large intestine, as the commonest eukaryotic organism detected in human stools. The taxonomy remains controversial, and not determined if a single species or multiple species exist in human and animal hosts. This parasite has been considered as a species complex comprising 17 subtypes, of which at least nine have been found in humans (Tan *et al,* 2002).

Forms of *B. hominis* in stools or in vitro cultures are vacuolar, granular, amoeboid, cyst, multivacuolar, and avacuolar forms. Infection is by cysts via fecal-oral route. The *B. hominis* has several modes of asexual division; binary fission, plasmotomy, schizogony and endodyogeny. *B. hominis* is an extremely prevalent form, which varies widely from one country to another country within the various communities within the same country or even city with symptomatic or asymptomatic infections (Stenzel *et al,* 1991).

Diagnosis depends on different laboratory techniques such as microscopy of the wet preparations stained with iodine or permanent stain with trichrome, the cultivation methods and the immuno-assay techniques. Molecular assays as PCR with sequenced-tagged-site primers, Nested PCR, conventional and real time- PCR were used (Tan *et al,* 2008).

In the present study, infection rate was 69% displaying an array of intestinal protozoa and helminthes diagnosed microscopically by wet mount smears. The most prevalent parasites was *B. hominis* (52%), followed by *D. fragilis* (19%), the least detected ones were *H. nana, E. histolytica* and *S. mansoni* (1% of each). Nascimento and

Moitinho (2005) in Brazil reported that 128/181 (70.7%) individuals were positive for intestinal protozoa and helminthes. *B. hominis* represented (26.5%) followed by *G. lamblia* (18.2%). These results agreed with El-Marhoumy *et al.* (2015) in Egypt who reported *Blastocystis* infection prevalence of 53%. Gashout *et al.* (2018) in Tripoli among 18,000 stool samples 15.7% had at least one parasite and the commonest parasite was *B. hominis* 41%.

A very low intensity of parasite in the specimens may lead to an increased number of false negative results diagnosed by wet mount smears. In clinical settings, false negative findings would affect the proper diagnosis, especially if the only microscopic examination was available. Moreover, the microscopic examination has disadvantages as the challenge posed by *Blastocystis* spp. diversity shape (Abou El Naga and Negm, 2001).

The present study showed that *B. hominis* was positive by light microscopy in 52%, by culture in 65% and PCR in 67% of cases. The PCR showed a better result compared to the microscopy and/or culture. Light microscopy is the convenient and least expensive method for examination and the widely used (Garcia, 2016).

The present study showed that culture was superior to microscopy in sensitivity. The culture proved to be reliable technique, but with more time consuming. Stensvold *et al.* (2007) recommended Jones' medium for the culture of *Blastocysts* spp. that allowed the preferential growth of the specific strains eliminating false negative cases. Kucsera *et al.* (2015) in Hungary by microscopy found that 16/78 (20.5%) had *Blastocystis spp.* compared to 26/67(38.8%) by cultures. But, Kukoschke *et al.* (1990) did not find any difference between microscopy and cultures methods.

Stensvold *et al.* (2007) reported that the molecular analysis was superior in *Blastocystis* spp. detection Parkar *et al.* (2007) recommended PCR of stool, compared to culture, with sensitivity largely affected by the DNA extraction used. Poirier *et al.* (2011) reported that PCR assays proved to be highly sensitive compared to the direct microscopy and culture methods. Roberts *et al.* (2011) showed that PCR was the most effective method for detecting *Blastocystis* in stool samples, but with disadvantages of being expensive, time-consuming, and need for specialized equipment. In the present study, 22 cases positive by microscopy were negative by PCR, which could be due to failure of DNA amplification possibly due to degradation of DNA processing or presence of contamination and inhibitors.

In the present study, SEM micrographs revealed different external topography of *B. hominis* covered by the surface coat which could be rough or smooth and extending between cells. Also, Zaman *et al.* (1999) reported that typical fibro-like surface coat projected beyond cells and many organisms were connected to one another by these fibrous structures. Binary fission and rosette formation as modes of reproduction agreed with Yamada and Yoshikawa (2012) and Zhang *et al.* (2012) and Zhang *et al.* (2007).

TEM micrographs showed both granular and vacuolar forms in same sample. Surface coat surrounded *B. hominis* different forms. The girdle-like cytoplasm contained several vacuoles, nuclei and mitochondria-like-organelles. Central body has few granules in vacuolar forms and filled with granules with different densities in granular forms. This agreed with Suresh *et al.* (2009) and Zhang *et al.* (2012). Chen *et al.* (1999) reported the development of cysts to vacuolar forms in human isolates and rat isolates in cultures. Also, Moe *et al.* (1999) found that the cells undergoing division developed from cysts into granular forms before becoming vacuolar in morphology.

Conclusions

Conventional PCR is the best method to detect *Blastocystis hominis* despite it is time consuming and more expensive than culture and light microscopy which is considered

more convenient and less expensive. The scanning and transmission electron microscopy showed the outer and inner features of *Blastocystis hominis*.

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Explanation of figures

Fig. 1: *B. hominis* from a stool sample; central vacuole with a thin peripheral rim of cytoplasm & multiple nuclei (Light Microscopy, Iodine Stain X 400).

Figs. 2 & 3: Vacuolar forms of *B. hominis* from Jone's culture media (saline & iodine)

Fig. 4: Gel electrophoresis stained with ethidium bromide showing results of PCR amplification of *Blastocystis* SSU rDNA from faecal samples. (1-11), (13,14): PCR positive samples showing distinct band at1800bp; (12): PCR negative sample; (N): negative control; (P): positive control. (M): molecular weight marker (100-bp DNA ladder).

Fig. 5: Detection of *B. hominis* in 100 stool samples by different methods.

Fig. 6: SEM of *B. hominis* from culture with rough surface coat.

Fig. 7: SEM of *B. hominis* form culture with smooth surface coat

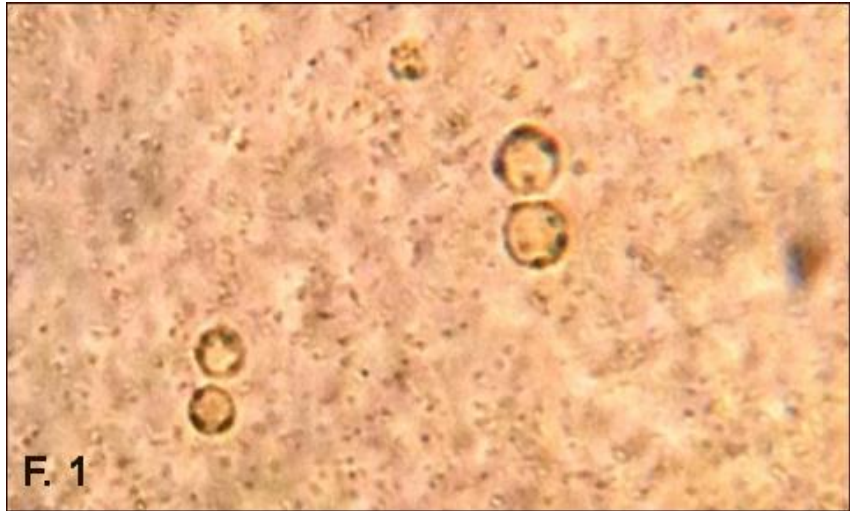
Fig. 8: SEM of *B. hominis* reproduction by binary fission from stool culture, showing dumbbell-like daughter cells formed by splitting of peripheral cytoplasm in center and both covered by a rough surface coat

Fig. 9: SEM from stool culture showing detachment of newborn cells of amoeboid form of *B. hominis* developing into 2 independent daughter cells and surface coat extending between them

Fig. 10: SEM of *B. hominis* reproduction observed in culture showing several daughter cells, and rosette formation

Fig. 11: TEM of vacuolar form of *B. hominis* with compressed cytoplasm surrounding central body partially granular. Peripheral rim of cytoplasm contains a nucleus and other smaller vacuoles (×8,000). Bar=2μm. N: nucleus, CV: central vacuole, SC: surface, V: vacuole, C: cytoplasm.

Fig. 12: TEM of granular form of *B. hominis* with compressed cytoplasm surrounding central body. Peripheral rim of cytoplasm contains mitochondria-like-organelle and other smaller vacuoles (×8,000). Bar=2μm. CV central vacuole, SC surface, V vacuole, MLO: mitochondria-like-organelle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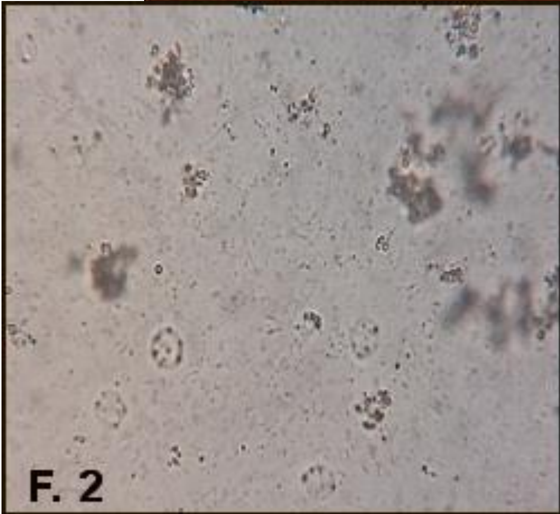
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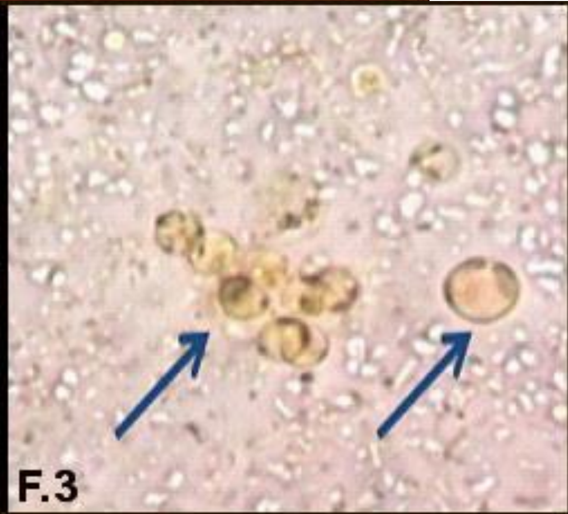
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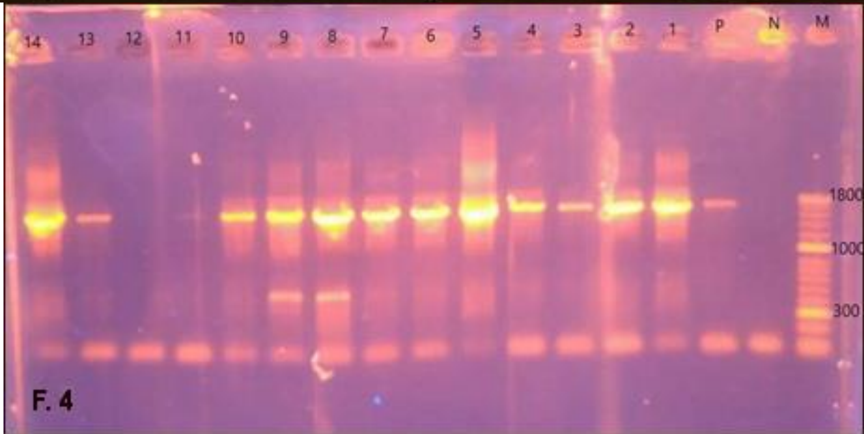
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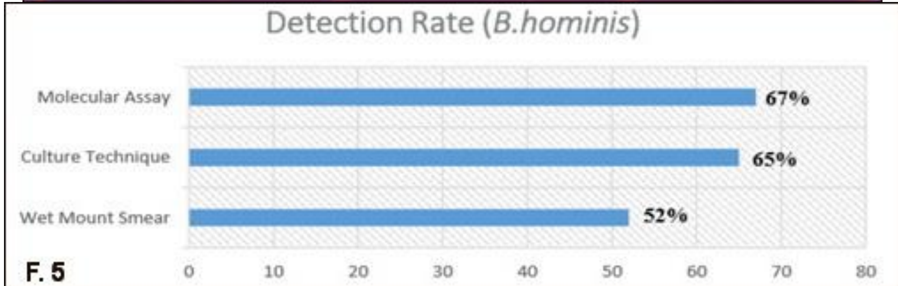
Detection Rate (B.hominis)
Molecular Assay
67%
Culture Technique
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Wet Mount Smear
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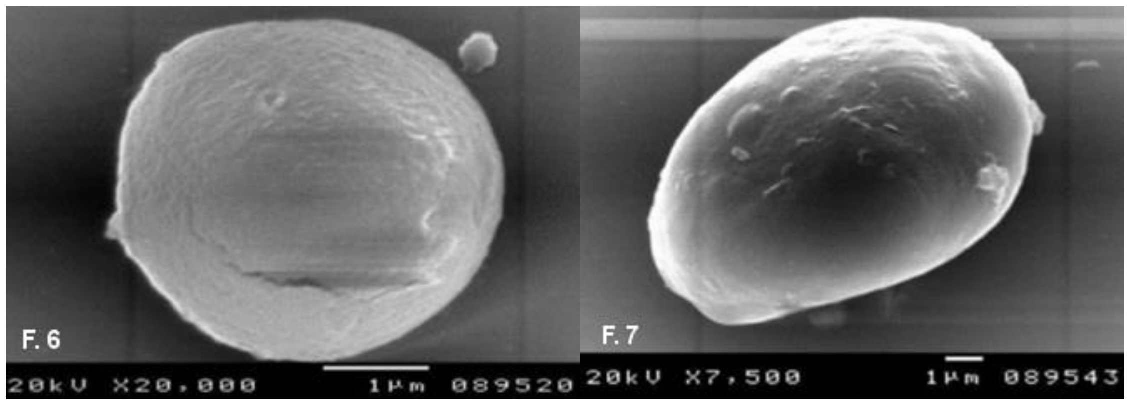
F. 5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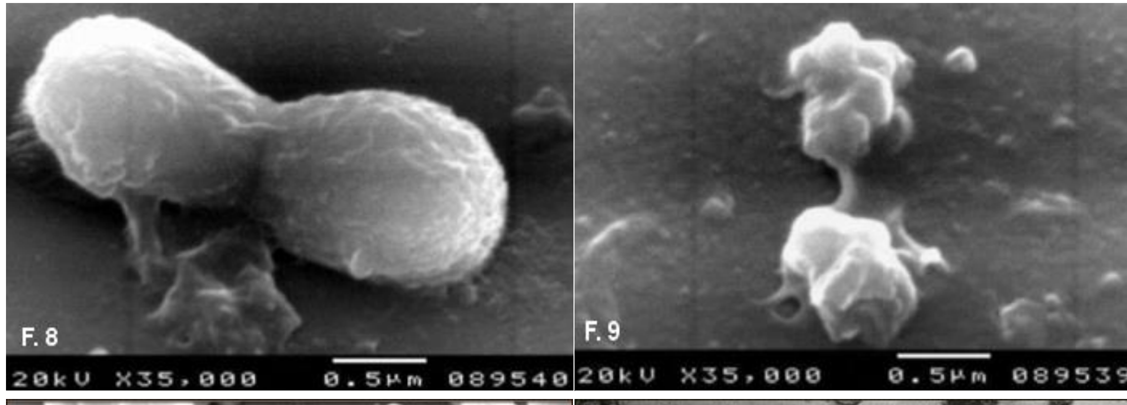
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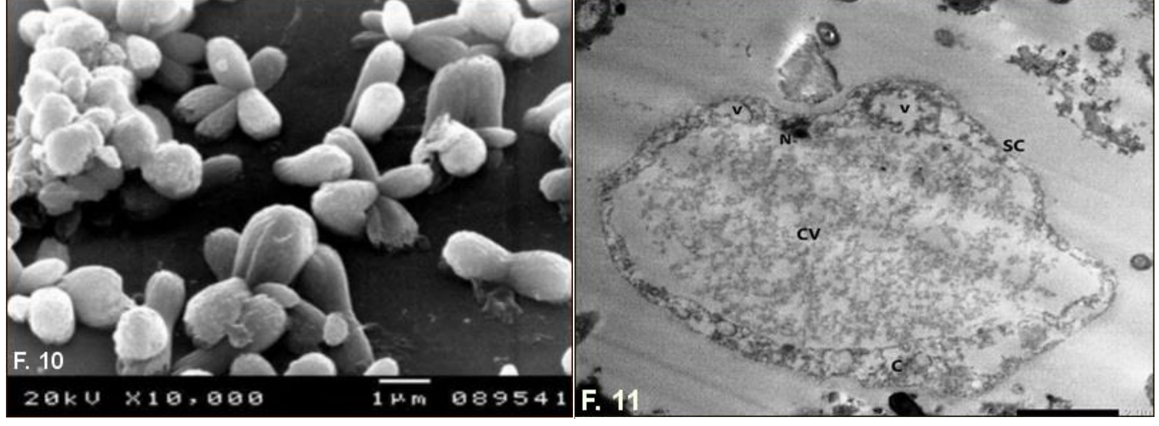
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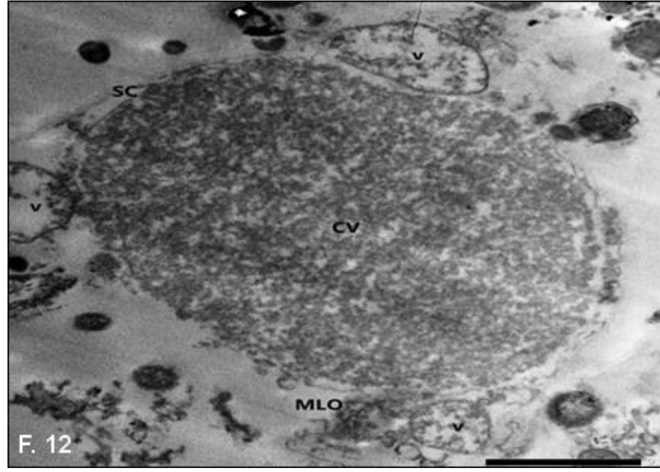
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