Plasmodesmata between Hyphal Cells of Geotrichum candidum

Abstract. *Electron-microscopic studies of three isolates of* Geotrichum candidum *from plants and one isolate from man showed the fine structure to be similar to other closely related, yeastlike fungi. Structures were observed that appeared similar to plasmodesmata present in higher plants.*

Geotrichum candidum (Link) ex Persoon emend Carmichael (*1*) is a facultative parasite capable of causing decay of certain fruits and vegetables and geotrichosis in man (*2–5*). Carmichael (*1*) classified *Geotrichum* sp. as imperfect, yeastlike fungi. The fine structure of several yeasts and yeastlike fungi, but not that of *Geotrichum* sp., has been reported (*3*). An electron microscope study was made of the fine structure of somatic mycelia of isolates of *G. candidum* from three citrus fruits and from one human. The plant isolates used were designated LA-2 (*5*), ATCC-7019 (*6*), and C-125 (*6*) and the human isolate CH (*5*).

The mycelia were grown on broth containing 20 g of dextrose, 133 ml of canned tomato juice, and the broth from 200 g of autoclaved, peeled Irish potatoes in 1 liter of distilled water. The medium was decanted into 250-ml Erlenmeyer flasks and autoclaved. The flasks were seeded from stock cultures of the test fungus maintained on potato-dextrose agar. Cultures were incubated at room temperature (22° to 32°C). Mycelial mats were harvested after 24 to 36 hours; killed and fixed for two hours in 1.0 percent nonbuffered potassium permanganate solution; dehydrated in an alcohol series; embedded in Maraglas for sectioning; and stained in 1.0 percent lead hydroxide.

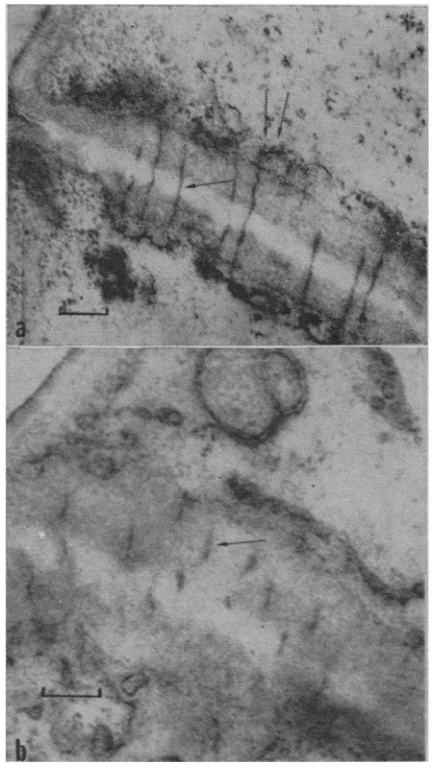

Figure 1. Electronmicrographs showing plasmodesmata (single arrow), in continuity with plasma membrane (double arrows), in between two fungal cells of plant isolate ATCC-7019 of *Geotrichum candidum* in longitudinal section (a) and oblique section (b) (1.0 cm = 0.1μ).

The ultrastructure of *G. candidum* is very similar to that reported (*3, 4, 7*) for closely related, yeastlike fungi, including species of *Histoplasma, Candida, Saccharomyces,* and *Blastomyces.* Distinct structures identical to the plasmodesmata found frequently in higher plants (*8*) were observed in the septa of the three plant isolates (Fig. 1), but not in the septa of human isolate CH. The failure to find these structures in isolate CH was attributed to difficulties in sectioning and staining caused by the presence of a thick, pellicle-like covering around the cells and by the thick cell walls of this isolate (220 to 290 μ compared to 60 to 120 μ for cell walls of plant isolates). The pellicle-like covering was not present in the plant isolates.

The plasmodesmata were observed to be connected with the plasma membranes (Fig. 1) in each of the adjoining cells. The fact that the plasma membrane retained its continuity with the plasmodesmata in sections in which the plasma membrane had pulled away from the septum, gave further evidence of this relationship.

If these structures, observed for the first time in this fungus, are comparable to plasmodesmata found in higher plants, then these structures would also function as protoplasmic bridges between hyphal cells in fungi. The presence of plasmodesmata is significant because entire septa are restricted to Phycomycetes and Hemiascomycetes, higher orders of fungi having protoplasmic connections through their septa (*9*).

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Auditory Sensitivity of the Monkey

Abstract. *Auditory thresholds for pure tones were determined in monkeys (three* Macaca irus *and one* Macaca nemestrina*) by the psychophysical method of constant stimuli after the animals had been trained by operant conditioning procedures. Their audible frequency range was found to extend from below 60 hertz to 40 or 45 kilohertz.*

The extent of the audible frequency range for sub-human primates has not yet been determined. Recent evidence (*1, 2*) indicates that the monkey is sensitive to higher frequencies than man, but the upper limit has not been established except for the marmoset (*2*).

In our experiment we attempted to find the upper limit of the audible frequency range for the macaque. To avoid the difficulties inherent in maintaining uniform free-field stimulation, we used a closed system with earphones mounted directly on the monkey's head (*3*). To measure sound pressure at the ear we inserted a probe tube (6 cm long, 1 mm in diameter) through the cushion of the earphone in such a way that the end of the tube was located directly in front of the phone at the entrance of the external canal.

The probe tube was connected to a condenser microphone (model 4134, Brüel and Kjaer) and cathode follower (model 2615, Brüel and Kjaer). The output of the cathode follower was

amplified and led directly to a wave analyzer (model 302 A, Hewlett-Packard). The phones (PDR 600, Permoflux) could reproduce frequencies of up to 50 khz with minimal harmonic distortion.

The present method may be more accurate and reproducible than existing procedures used on the awake, intact monkey (*1, 2, 4*). However, at the higher frequencies there is probably some error in measurement caused by standing waves in the probe tube and in the ear canal. We feel that the most serious problem in studies of auditory thresholds are related to the presentation and measurement of stimulation by high frequency tones.

The subjects, three young male *Macaca irus* (M-2, -3, and -6) and one adult male *Macaca nemestrina* (M-5) were kept in restraining chairs (*3*) for the duration of the experiment. Experimental sessions were conducted in a double-walled, sound-proofed room (Industrial Acoustics); the programming and recording equipment were located outside the room. During a session the subjects were further restrained to prevent head movement so that head phones could be fitted over the openings of the ear canals and kept in place for the duration of the session.

The monkeys were conditioned to press a telegraph key (A) for food reinforcement (190 mg whole diet, banana-flavored pellets, Ciba) in the presence of a pure tone (1 khz at about 100 db re 0.0002 dynes/cm^2) binaurally presented. After conditioning, the tone was turned off, and reinforcement was withheld when the animal responded by pressing key A. A second key (B) was then added. Pressing key B turned on the tone for 3 seconds during which time a response on key A was reinforced with food. The tone was scheduled aperiodically in such a way that only occasional responses on key B produced it. The average interval between presentations of the tone was about 15 seconds. Pressing key B during the tone had no effect; pressing key A in the absence of the tone resulted in a 3 second time-out from the experiment. The time-out was used as a mild form of punishment (*5*). During the time-out the animal was in effect disconnected from the experiment, and the programming equipment stopped.

After the animals had been trained, their thresholds were estimated by decreasing the intensity of the tone until a response on key A failed to occur.

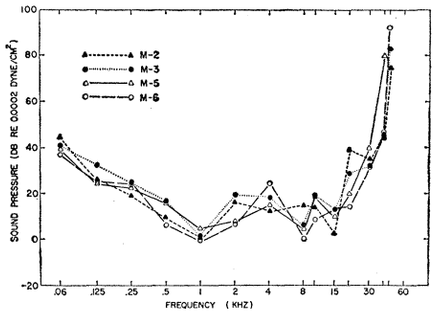

Fig. 1. Auditory threshold functions for the four monkeys from this experiment.

Five intensities above and below the estimated threshold were selected. These intensities, at intervals of 10 db, were presented to the animal in a mixed order; each intensity was presented ten times for a total of 50 trials (one animal was given 20 trials at each intensity). The absolute threshold was calculated on the basis of the number of times the monkey pressed key A in response to each intensity. The frequency with which key A was pressed was a function of the intensity of the stimulus, and the threshold was defined as that intensity which produced a response on key A 50 percent of the time that it was presented.

Thresholds were calculated in this manner at 13 frequencies ranging from 60 to 45,000 hz. Subjects were tested at 2 different frequencies each day. Each subject was tested until, for two successive sessions, the thresholds were not more than 5 db apart at any frequency.

The results of the test are shown, for each individual monkey, in Fig. 1. Each point on the curve represents the average threshold at each frequency in the final two sessions in which it was given. Our data show that the monkey's hearing is most sensitive to a frequency of 1 khz. For the most part, our subjects were only slightly less sensitive to 8 and 15 khz. There is a definite decrease in sensitivity of all subjects to frequencies of 2 and 4 khz; this finding has been reported previously (*1, 2*).

In general, the sensitivity of our monkeys (see Fig. 1) to the frequencies between 60 hz and 30 khz closely resemble thresholds obtained at these frequencies by previous investigators. The differences that do exist are not great and may be due to the differences in training procedures, but they are more likely a result of the closed system (earphones) used in this experiment for presentation and measurement of sound.

One monkey, M-5 (*M. nemestrina*), showed a sharp decrease (40 db) in sensitivity to frequencies between 30 and 40 khz. We were unable to evoke a key A response from this animal at 45 khz at a sound pressure level (SPL) of almost 100 db. The other subjects (*M. irus*) showed an equally sharp decrease in sensitivity to frequencies between 40 and 45 khz. No key A response could be evoked from these animals at 50 khz at about 95 db SPL. Because our sample was small and because the *M. nemestrina* was the oldest animal in our study, species comparisons are hardly meaningful. It is clear, however, that the macaque can hear frequencies ranging at least one octave above those heard by man.

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Spermatophore Web Formation in a Pseudoscorpion

Abstract. *The male pseudoscorpion* Serianus *sp. n. deposits spermatophores without mating, but only when females are present. It marks a path to the spermatophore with silken threads produced in an endodermal gland, which is homologous to the rectal pocket in males and females of other species and in the female of the same species.*

Pseudoscorpions transfer sperm in one of two ways. The more advanced families, Chernetidae and Cheliferidae, have a pairing behavior in which they perform mating dances (*1, 2*). The Chthoniidae, Neobisiidae, and Cheiridiidae do not mate. Even when females are not present the males of these fami-