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THE TEMPERATURE-DEPENDENT EMISSION OF LOW FREQUENCY SOUND BY MOTILE CULTURES OF THE CILIATE TETRAHYMENA THERMOPHILIA

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When a sensitive condenser microphone is appropriately sealed and immersed into a motile suspension of Tetrahymena, a characteristic sound spectrum can be recorded. The spectrum of cells cultured at 25°C and measured at 20° contains three main components, centred around 40, 55 and 80 Hz. Both the intensity and distribution of the sound emission are altered when the cells are cooled to 12° or warmed to 33°C. No such sound emission is detectable from suspensions of sessile organisms.

Recently, evidence has been presented that suggests that the motility of Tetrahymena is correlated with membrane fluidity (1,2). In order to corroborate and subsequently utilise this finding, further measurements of the swimming velocity will have to be made. Unfortunately any accurate measurement of the swimming velocity entails tedious manual observations (2), so a search was initiated for a more convenient method. Two possibilities presented themselves, the first being to measure the autocorrelation function of a cell suspension, utilising scatterec laser light (3). The second and simpler possibility was to see whether the ciliary beat of Tetrahymena could be monitored using a suitable sound (pressure) detecting system. Intuitively, it would be expected that the power stroke of the ciliary beat, and perhaps also the accompanying metachronal wave, would set

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up a hydrostatic pressure wave whose frequency would be related in some manner to the ciliary beat frequency. This method of measurement, if succesful, would be expected to be more informative than that obtained by autocorrelation analysis, which could only yield information about the velocity of the whole cell. Described below are experiments which demonstrate that sound-waves can be detected in cultures of motile Tetrahymena, and examples are given showing that the sound-spectra are temperature dependent.

MATERIALS AND METHODS

Cultures of T. thermophila Chx-2/Chx-2 (cysens, IV), hereafter referred to as Tetrahymena, were cultured at 25°C in a Bacteriological peptone yeast extract medium supplemented with 36 µM $FeCl_3$.

For the acoustic measurements, cells were concentrated by centrifugation at 240 x g, and placed inside a low-form measuring cylinder of 3 cm internal diameter. The cylinder was then placed in an insulated polystyrene container (Figure 1). A Brüel & Kjær 1" condenser microphone Type 4145 with a Type 2627 preamplifier was mounted in a heavy brass collar which fitted tightly over the

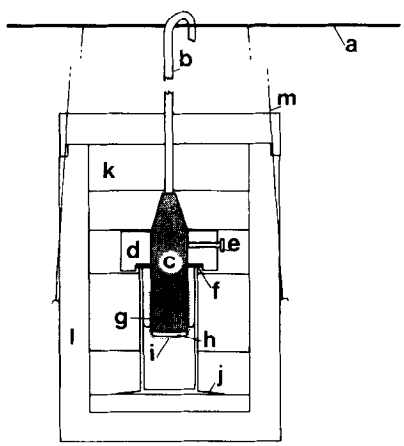

Figure 1

Arrangement for detecting sound emission from Tetrahymena. The cell culture in a glass cylinder (j) is placed in a polystyrene container (l), and kept in place with foam rubber rings (k). Two layers of Parafilm (i) are stretched tightly round the protection grid (h), microphone (g) and preamplifier housing (c) to prevent damage to the condenser diaphragm. The microphone is clamped into a brass collar (d) by means of a screw (e), and immersed in the cell suspension; the rubber gasket (f) ensures an elastic contact. The whole assembly together with the preamplifier cable (b) is suspended by a thin wire (m) from a taut steel wire (a) hung across an anechoic chamber. See also Methods.

top of the cylinder. A screw enabled the level of immersion of the microphone in the solution to be accurately adjusted. Before immersion, the diaphragm and vent-holes of the microphone were carefully sealed by the stretching two layers of Parafilm over the protection grid and up the sides of the entire housing. The microphone was immersed 3-4 mm into the cell suspension. The whole assembly was suspended from a steel wire stretched across a large anechoic chamber maintained at 20.0°C. Frequency spectra were recorded, following a 5 min settling down period, using a Brüel & Kjær Fourier Transform frequency analyzer, Type 2033. The temperature of the cultures was measured immediately following the recording of a spectrum using a Mettler TM 15 thermistor.

RESULTS AND DISCUSSION

In the initial phases of the experiments, unsuccesful attempts were made to record sound emission from the cultures using piezoelectric hydrophones or transducers clamped to the exterior of the container containing the cell culture. Condenser microphones have a greater intrinsic sensitivity than hydrophones, but when the sound source is in the aqueous phase, hydrophones are to be preferred, as there is an approximately 25 dB (re 20 μ Pa) loss at the water-air interface (see Ref. 4 for a discussion). By placing a thin membrane over the protection grid of the condenser microphone, a small volume of air is trapped in front of the diaphragm, which thus acts as a coupler between the aqueous medium and the diaphragm, and eliminates the above-mentioned 25 dB loss. Using this set up, reproduceable frequency spectra were obtained within 3-10 min if the cell density was above 3×10^5 cells/ml. Scans in the range up to 10 KHz were made initially, but the region of acoustic activity was always seen in the 25-100 Hz region. A typical spectrum, together with the background spectrum of medium without cells, is shown in Fig. 2. In the background spectrum, a number of large peaks are seen in the frequency range 1-25 Hz; these are caused by oscillations of the anechoic chamber and the suspending wire. There is also a strong peak at 50 Hz, (together with higher harmonics) due to the mains power supply. Above 25 Hz, the background spectrum is relatively

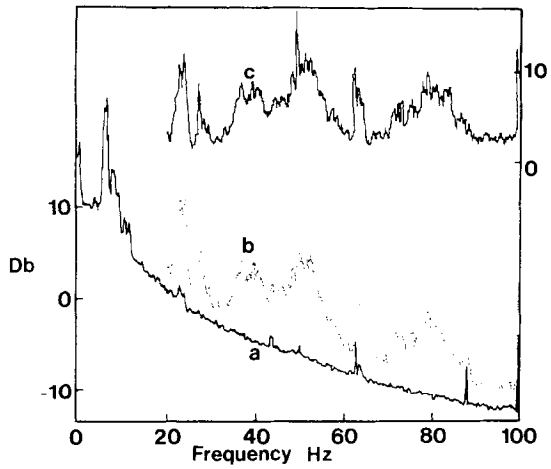

Figure 2

a. Frequency spectrum of culture medium. Sweep width 100 Hz, 400 data points, 654 scans (2 sec cycle time), linear averaging, Hanning weighting, 20°C.
b. Frequency spectrum of Tetrahymena cultured at 25°C, 3.7 x 10^5 cells/ml. As in a. but 315 scans.
c. Difference spectrum obtained by subtracting spectrum a from spectrum b.

quiet, so that spectra from the Tetrahymena culture could be acquired within a few minutes of leaving the chamber. It can be seen that there are three main peaks in the spectrum, centred around 40, 55 and 80 Hz. These complex peaks could for example originate from different fields of cilia, e.g. the oral cilia (including the undulating membrane) and the cortical cilia. The distribution of frequencies about the means could be accounted for by the variation expected within any population.

Unfortunately, very little is known about the frequency of the ciliary beat in Tetrahymena, although in ciliates as a whole, beating rates lie within the range 10 to 100 Hz (5,6). In Paramecium, the velocity of metachronal wave transmission and the beat frequency are reduced as the temperature is lowered (7). Since the swimming velocity of Tetrahymena decreases with temperature (1,2) it seems likely that here too, the beat frequency is temperature dependent. For these reasons, frequency spectra were recorded from Tetrahymena suspensions at two dif-

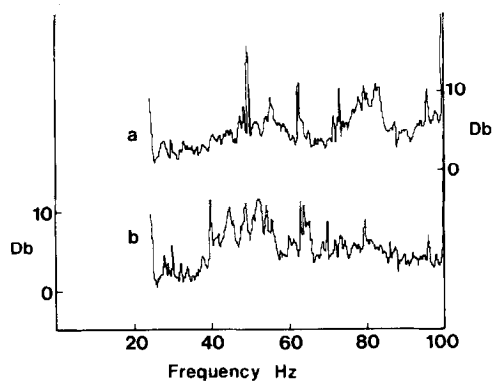

Figure 3
a. Difference spectrum of Tetrahymena cultured at 25°C, 3.5 x 10^5 cells/ml, but warmed to 32°C. Conditions as in Figure 2, but 280 scans. Spectrum 2a was used as the reference.
b. As in 3a., but cells cooled to 12°C, and 458 scans.

ferent temperatures (Figure 3). The difference spectra at 12° and 32°C differ noticeably from that observed at 20°C (Figure 2). At 32°C the 80 Hz band remains unaffected, while the 40 Hz band is markedly diminished and the 55 Hz band has shifted to a slightly higher frequency. At 12°C, the 80 Hz band has almost disappeared while the 40 and 55 Hz bands are relatively unchanged compared to the 20°C spectrum. The changes observed in these and other experiments are complex, and their interpretation will require continued studies.

In order to be certain that the frequency spectrum was correlated with motility, spectra were also recorded from cells in which the cilia had been stripped off by passing the cells repeatedly through a Pasteur pipette. The spectra of these cultures were identical to that shown for the culture medium alone (Figure 2). Furthermore, when the cell density of a motile suspension was increased 4 times, the intensity over the whole spectral range was increased by approximately 6 dB, in agreement with theoretical expectations (8,9). On this basis, it can be calculated that each Tetrahymena has a sound emission corresponding to between - 45 and - 50 dB (re. 20 μPa).

CONCLUSION

The data presented above demonstrate for the first time that sound (pressure) waves can be recorded from a motile cell population. Furthermore, the changes in frequency and intensity of the signals with temperature, together with the total absence of signal in immotile cultures suggests that the sound spectrum is directly correlated with ciliary activity.

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