Sharp Resonances in Yeast Growth Prove Nonthermal Sensitivity to Microwaves

W. Grundler
Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg, Federal Republic of Germany

and

F. Keilmann
Max-Planck-Institut für Festkörperforschung, D-7000 Stuttgart 80, Federal Republic of Germany

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Microwaves near 42 GHz are found to influence the growth of *Saccharomyces cerevisiae*. The growth is measured photometrically in stirred aqueous culture. The microwave effect occurs and saturates above a threshold intensity $\ll 10$ mW/cm^2, excluding any explanation based on microwave heating. A surprisingly strong frequency dependence is observed, with resonances as narrow as 8 MHz. These results confirm the existence of a nonthermal resonant microwave sensitivity in biology; they suggest yet unknown tuned systems triggering yet unknown biological actions.

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The existence of a nonthermal microwave sensitivity in biological systems has not been generally accepted. Experimental evidence has been scarce. Thus low-intensity millimeter waves were reported to affect microbial growth and metabolism[1-6] or to reduce x-ray sensitivity of bone marrow cells in mice.[7] Not all of these reports, however, can convincingly exclude that a slight heating might have caused the observed effects. Theoretically, nonthermal microwave effects were conjectured by Fröhlich.[8] He discusses a thresholdlike behavior in the metabolic excitation of large-amplitude vibrations; this leads to a storage of energy and a resonant sensitivity to external radiation.[9] Here we report evidence of nonthermal resonant action of millimeter microwaves on the growth of yeast cultures, corroborating our earlier findings.[5]

The experiment emphasizes high-frequency stability in order to resolve the unexpectedly narrow resonances; it furthermore provides the use of two greatly differing irradiation geometries to rule out directly artifacts from the microwave system. The procedure[10] used diploid, homozygot, and isogene wild type *Saccharomyces cerevisiae* grown on agar for three days at 30 °C, then stored at 4 °C. Liquid suspensions with starting concentrations near 3×10^5 cells/ml were held in glass cuvettes equipped both with mechanical stirrers and with submersible Teflon antennas for coupling in microwaves.

One such cuvette was placed in the measuring arm each of a Beckman Acta CIII and a Beckman 24 double-beam spectrometer set at 550 nm, while the reference cuvettes in both instruments were filled with plain growth medium. The optical-density output signals V_{OD} were amplified in logarithmic amplifiers and continuously recorded as $\ln V_{OD}$ vs time t, to give straight lines in case of exponential rise of the signals. This proved to be the case during a growth period of roughly 3 h, so that an exponential growth rate $\mu = t^{-1}\ln V_{OD}$ could be read off the plots with $\pm$ 1% uncertainty.

Care was given to temperature stabilization and power measurements. The cuvette housings were thermostatted with flowing water at 30.7 $\pm$ 0.1 °C. The microwave system included a 1-m-long waveguide which coupled source to sample via an isolator, two directional couplers to monitor forward and backward running power, and finally an impedance transformer adjusted to minimize reflections. Standard procedures ascertained that standing-wave resonances of the system did not exhibit spectral widths narrower than 100 MHz, confirming expectations.[11] Continuous monitoring of the power incident on the sample was supplemented by a continuous calorimetric measurement of the microwave power absorbed by the sample, as already used by us,[5] whereby the temperature of the sample suspension is increased by 0.016 °C per milliwatt absorbed power. This proved that (90 $\pm$ 10)% of the incident power was absorbed in the sample. The price to pay for this rather direct power measurement is that the irradiated sample's temperature was not fixed at 30.7 °C but had an overtemperature varying from 0.16 to 0.4 °C for the usually applied power range from 10 to 25 mW. This results in a small thermal effect (Fig. 2, dashed) on the growth rate, increasing μ by 2.7% per degree Celsius near 31 °C as established separate-

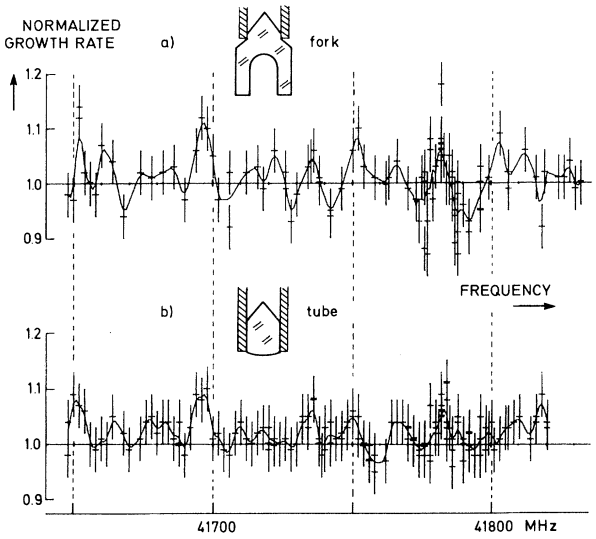

FIG. 1. Microwave effect on yeast growth vs frequency with use of either (a) fork or (b) tube antenna.

ly.[12]

Two different submerged antennas were used to apply the radiation to the yeast cells through the suspension (note that the microwave power penetration depth is 0.2 mm as a result of water absorption). One was the fork-shaped Teflon body designed to give a large radiating surface of 8 cm^2 (similar to the one used in Ref. 5, Fig. 5). This structure sustains a complex microwave field distribution; local intensity variations along the irradiating surface of up to a factor of 3 were estimated[13] from the known Teflon/water reflection coefficient. Specific laser-interferometric measurements[13] enabled us to record the temperature distribution at the boundary *in situ*, with 1-mm spatial resolution. The result was indeed that sizable irradiation occurred on any part of the surface, with no prominent "hot spots." Quantitatively, the conclusion is that for an applied power of 40 mW the maximum possible temperature difference between any two points in the stirred suspension is 0.02 °C.[14] The second antenna has a simple tubular form; the submerged metallic circular waveguide is plugged with a Teflon stopper with slightly curved surface to avoid a trapping of air bubbles (inset of Fig. 1). The microwave fields are rather homogeneous but the radiating surface is relatively small (0.4 cm^2) giving the stirred yeast cells a reduced chance of passing through the irradiation region, so that smaller effects are expected.

The growth rates vary, in the absence of irradiation, within ± 10% of the mean. The relative fluctuations between simultaneously growing cultures stay, however, within ± 4%, which led us to use one spectrometer for irradiation to obtain μ_x, the other for control to obtain μ_0, and to plot normalized growth rates $\bar{\mu} = \mu_x/\mu_0$. Usually the irradiation was turned on after 1 h of exponential growth and μ_x determined from the slope thereafter. Each growth experiment is represented in Figs. 1 and 2 by a single point and an error bar of ± 4%. On the frequency scale an error bar of ± 1 MHz is assigned because the irradiation frequency shows residual modulation up to 0.5 MHz while being electronically stabilized (marginal phase locking) to an absolute accuracy of ± 0.1 MHz.

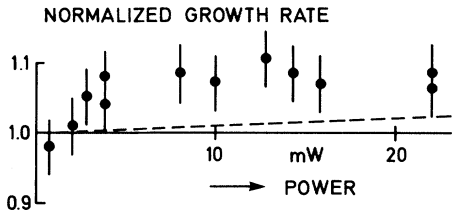

FIG. 2. Microwave effect vs power at fixed frequency (41 782 ± 1 MHz, fork antenna). Dashed: contribution of thermal effect.

The results (Fig. 1) show that microwave effects of about 10% are obtained, both in the directions of enhanced and of reduced growth. The effects obtained with the tube antenna do not seem to be smaller. Hence it appears that a reduced microwave intensity available quite far from the radiating surface suffices to cause the effect. That a small intensity threshold ($\ll$ 10 mW/cm^2) should exist above which effects stay constant

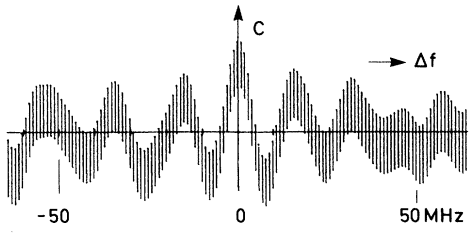

FIG. 3. Central part of cross correlation of yeast response spectra Figs. 1(a) and 1(b), with $C(\Delta f) \sim \int \bar{\mu}_a(f)\bar{\mu}_b(f-\Delta f)df$. The significant maximum at $\Delta f = 0 \pm 1$ MHz proves together with the mirror symmetry around this point that both spectra *a* and *b* agree and reproduce the resonance positions to within ±1 MHz, while the width of the resonances at half height is 8 MHz.

(Fig. 2) was conjectured[8] and observed before, in mice.[7]

Statistical examination[10] indicates a negligible probability that either spectrum *a* or *b* could be a chance sequence only of a frequency-independent microwave effect. Also, the yeast response spectra obtained with both antennas have great resemblance, e.g., maxima occur at 41697 and 41782 MHz. A cross-correlation analysis (Fig. 3) clearly proves this to be true, i.e., both experimental series probe a common feature which is a spectrally selective response inherent in the yeast cells.

Further inspection of Fig. 3 reveals also significant correlation at a frequency offset of 16 MHz, which demonstrates for the first time that the narrow resonances are accompanied by sideband satellites.

In conclusion we confirm a nonthermal resonant microwave sensitivity in the case of yeast. Our result enhances the weight of reports on other systems[1-7] and poses the question of generality of this sensitivity in biology. Uncovering its origin will provide a fascinating interdisciplinary task.

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[14]Note that the possibility of microscopic hot spots on cellular or subcellular scales can be rigorously ruled out from heat conduction arguments (Refs. 11 and 13).