Effect of Millimeter-Wave Irradiation on Growth of *Saccharomyces cerevisiae*

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Abstract—**Cultures of *Saccharomyces cerevisiae* were exposed for 4 h to millimeter waves in three frequency ranges between 41.650 and 41.798 GHz. The irradiation frequency was stabilized to within ±50 Hz. The temperature difference between irradiated and sham-irradiated samples was maintained to within ±0.01°C. Growth was measured optically during the irradiation, and viability counts were done at the end of the irradiation. At least three experiments were performed at each of 15 frequencies.**

No differences larger than ±4 percent were detected in the growth rates at any of the selected frequencies. Such differences were not significant at the 95 percent confidence limit. Results obtained with plate counts correlated favorably with the optical absorbance data. While our data are in contrast with those reported from other investigators, these experiments support conclusions of our previous studies, and of some other investigators, showing that, under strictly controlled conditions, no statistically significant nonthermal effects can be induced by millimeter-wave irradiation of a variety of prokaryotic and eukaryotic cells.

INTRODUCTION

SINCE 1968 several investigators have reported a variety of biological effects induced by irradiation with millimeter waves (mm-waves). Many have further claimed strong dependence on the irradiation frequency. Representatives of these findings include effects on growth rates of *Rhodotorula rubra* [1], *Saccharomyces cerevisiae* [2]–[6] *Escherichia coli* [7], [8], *Candida albicans* [1], [9], reduced viability in *Sacch. cerevisiae* [10], protection of rabbit bone marrow cells from X-ray damage [11], increased colicin [12] and lambda phage induction in *E. coli* [13], and puffing of giant chromosomes of *Acricotopus lucidus*, an insect [14].

Some reports indicated changes in the magnitude of the effect when the frequency was shifted by a few megahertz. Furthermore, some reports were characterized by the low power densities needed to induce the effect; a power density as low as 10 $\mu W/cm^2$ was reported to effect colicin induction in *E. coli* [12]. An extensive review of the published literature can be found in [15].

Unfortunately, many of the above mentioned reports lack essential details of the experimental procedure and data, making independent duplication or evaluation quite difficult. Some of tnem, however, have been independently repeated, mostly with negative findings. Some of the negative experimental results include: no effects on growth rate of *E. coli* [16] and no changes in viability of *Sacch. cerevisiae* [17]. No evidence of cytological effects as determined by electron microscopy [18], and in protein synthesis [19] were observed in BHK-21 cells exposed to 202 frequencies in the *E*- and *U*- band at various power densities. Finally, no statistically significant effects were found on colicin induction [20], [21], on mutation rates of *Salmonella typhimurium* or on induction of lambda phage in *E. coli* by *U*- and *E*-band mm-wave irradiation [22], [23].

A strong dependence on irradiation frequency of the growth rate of yeast suspension has been reported independently by two research groups. Devyatkov [1] reported increases up to +30 percent in the growth rate of *R. rubra* exposed for 15 h at 7.18 mm (41.783 GHz) and a decreased growth rate of up to −40 percent when exposed at 7.19 mm (41.725 GHz). However, no indications were given about experimental procedures such as irradiation conditions, handling of the controls, temperature control and measurement, frequency stability, and power density.

Grundler, Keilmann, and co-workers [2], [6] have also reported that the irradiation of *Sacch. cerevisiae* in the 41.650–41.800 GHz range, showed both growth enhancement (up to +15 percent) and growth inhibition (down to −13 percent) frequency regions. Frequencies exhibiting significantly different effects were reported to be only 8 MHz apart. The data suggest that the yeast suspensions showed a sensitivity to frequency changes of less than 1 part in 5000. Due to this reported frequency dependence, this phenomenon has been termed a "resonant effect" [2]–[6].

Although such reported frequency sensitivity has stimulated interest in the possible mechanism and site(s) of action of mm-waves [18]–[23], [24], [25], it is essential to establish the validity of these observations and their interpretation. In the following we report on our experiments designed to study further the possible effects of mm-waves on the growth of *Sacch. cerevisiae*.

MATERIALS AND METHODS

A. Description of the Irradiation Experiment

The organism used in this study was obtained from the University of Utah stock collection, grown on Sabouraud

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glucose agar (Neopeptone® 10 g/l, glucose 40 g/l, Bacto agar® 15 g/l) stored at 4°C and passed every month. From these cultures, organisms were inoculated in Sabouraud glucose broth (Neopeptone® 10 g/l, glucose 20 g/l, pH 5.6) and incubated overnight at 32°C on an orbital shaker (40–50 rpm). The overnight culture was diluted 1:400 and incubated at 32°C for 2 h on an orbital shaker before loading each of two irradiation circuits with 2.5 ml of the suspension. The irradiation and sham irradiation were started after another hour of incubation in the circuits so as to irradiate the yeast during the log phase of growth. The temperature difference between irradiated and sham-irradiated suspensions was maintained within ±0.01°C using a nichrome heater placed under the sham-irradiated sample holder. The suspensions were recirculated with a peristaltic pump LKB 2115 with a flow rate of 7 ml/min. The experimental temperature was 32.0 ± 0.1°C. The temperature coefficient of the growth rate was measured between 30 and 34°C and was 0.024 h^{-1}/°C. The mean growth rate at 32°C was 0.595 h^{-1}, corresponding to a duplication time of 70 min.

The irradiation time was 4 h with power in the waveguide set at 20.0 ± 0.5 mW. Due to the tapered design of the sample holder, the power coupling of mm-wave radiation to the suspension was larger than 99 percent as estimated from reflected power measurements. Even though we used continuous wave (CW) microwaves, circulating the suspension did, in fact, provide a modulation of sorts with a period of 30 s and a duty cycle of 0.06.

Small flow-through cuvettes were placed within the recirculating circuits to allow turbidity measurements. For both the irradiated and sham-irradiated samples, triplicate sets of data were collected at the beginning of the irradiation and hourly thereafter. All the data collection, storage, and analysis was performed with an IBM personal computer. Absorbance values at the observation time intervals were analyzed with a regression analysis program to fit an exponential curve. The output of the program gave the estimated mean values of growth rates μ_i and μ_c for irradiated and sham-irradiated suspensions. The program also indicated the 95 percent confidence limits of the estimated growth rates for each experiment. A typical exponential growth curve, measured optically, for an experiment without either mm-wave radiation or compensatory electric heating is shown in Fig. 1.

Plate counts were done at the end of the irradiation experiment using three 0.2 ml aliquots from each suspension. After tenfold serial dilution, the suspensions were plated in quadruplicate and colonies counted after 48 h of incubation at 32°C. Dilutions were chosen to give approximately 200 colonies per plate.

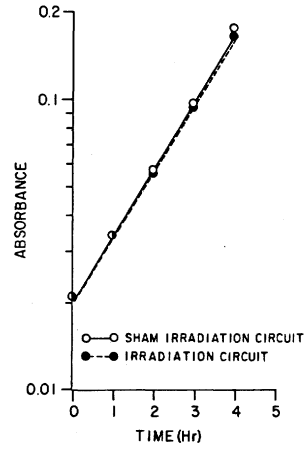

Fig. 1. A typical growth curve of *Saccharomyces cerevisiae* suspension measured optically in the absence of mm-waves. For this particular experiment the growth rate of the "sham-irradiation" was 0.531 h^{-1}, while for the "irradiation" circuit it was 0.522 h^{-1}. The 95 percent confidence limits were about ±0.015 h^{-1} (±2.5 percent). The points represent the average of three measurements taken at the indicated time intervals; the lines represent the fitted curves calculated with an exponential regression program. The cell concentrations at the start and at the end of an experiment were in the 6.5 × 10^5–1.1 × 10^6 and 7 × 10^6–1.2 × 10^7 cell/ml ranges, respectively. The time intervals and dilution factors were chosen so as to do experiments only during the log phase of the yeast growth and to use the linear response region of the photometric system.

B. Microwave System

As mentioned previously, the main feature of a so-called "resonance effect" is its strong dependence on the irradiation frequency. Therefore, some of the basic requirements for observing such an effect should be 1) high frequency stability within a single experiment, 2) high precision in the frequency resetting from experiment to experiment, 3) high accuracy in the absolute frequency determination, and 4) low noise and narrow bandwidth of the source. After testing various mm-wave sources such as free running klystrons and IMPATT diodes, we chose a klystron, Varian model VA302-BT, phase-locked by a source-locking microwave counter, EIP model 578. By using a highly stable quartz crystal enclosed in a temperature controlled oven, the rated frequency stability of the counter was ±50 Hz for a center frequency around 40 GHz. This stability was for at least 4 h, the typical length of an experiment. The absolute frequency could be measured with up to 11 significant digits. The rated aging of the reference quartz crystal is claimed by the manufacturer to give a long-term accuracy (1 year) of the absolute frequency of less than ±8 kHz. The residual half-power bandwidth was 40 kHz when phase-locked, as measured with a Tektronix 491 spectrum analyzer.

The CW power in the waveguide was measured with a thermistor, Hughes model 4489H, connected to a power meter, Hewlett-Packard model 432A. A variable attenuator was inserted before the thermistor to cut off, from time to time, the microwave power to the thermistor, therefore allowing correction for possible thermal drift of the thermistor. The sample holders were mounted at a 6° angle directly on the waveguides (Fig. 2) thereby providing enough tapering to ensure a measured power coupling of about 99 percent. It was confirmed during preliminary tests that the reflected power was always less than 1 percent, regardless of the irradiation frequency. The fixed geometry of the sample holder did not require routine measurement of the reflected power. A complete block diagram of the microwave circuit is shown in Fig. 3.

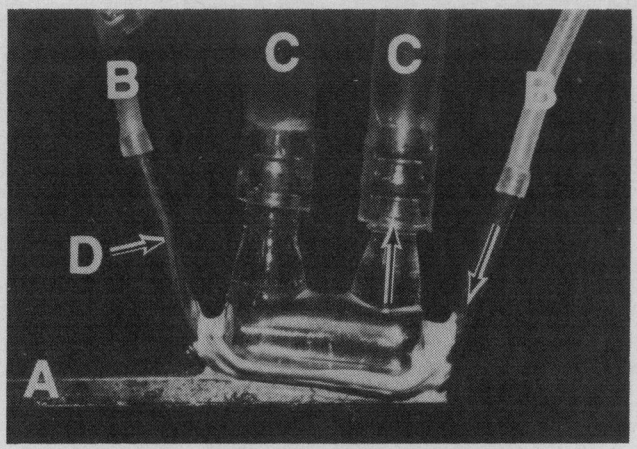

Fig. 2. Sample holder used for irradiation. The Teflon® coated thermocouples were inserted in the suspensions through holes in the silastic rubber tubing. *A*—WR-19 waveguide; *B*—cell suspension circuit; *C*—water jacket circuit; *D*—thermocouple for the temperature measurement of the sample at the exit of the sample holder. Another thermocouple was placed also at the sample holder inlet. The directions of flow are indicated by the arrows on the circuits. The nichrome heater was placed between the waveguide and the sham-irradiation sample holder.

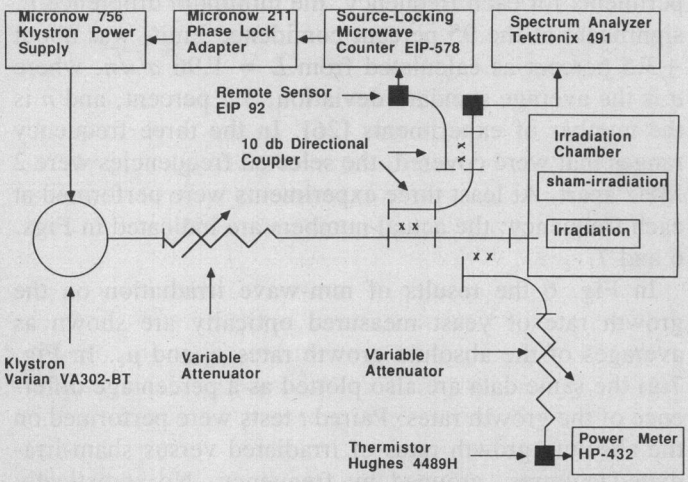

Fig. 3. Block diagram of the microwave system. The section necessary for the reflected power measurement was removed during the actual experiments.

C. The Irradiation Chamber

The chamber was made with an aluminum box lined with polyurethane foam. On the bottom of the chamber we placed a copper tank in which temperature controlled water was recirculated at the rate of 10 l/min. The water was circulated from a water bath kept at 32.0 ± 0.1°C by a YSI 72 proportional temperature controller and a solid-state cooler (Whirlpool). The waveguides with the sample holders were bolted on the copper tank. To obtain a uniform temperature distribution we found it essential to place an electric fan inside the chamber. The 60 Hz magnetic field generated by this fan did not differ from the background intensity, 0.03 G, at either the sample holder or the cuvette location. A top view of the irradiation chamber is shown in Fig. 4. The glass sample holder had a volume of 0.25 ml (2.5 × 0.5 × 0.2 cm) and was connected to the peristaltic pump with silastic rubber tubing (1.3 mm i.d.). Water jackets were placed around the glass sample holders to improve the thermal exchange. Water for the water jackets was circulated at a rate of 1 l/min, from the same water bath supplying the copper tank. Teflon® coated thermocouples (Bailey-Sensortek IT-18) were placed in the suspensions at the inlets and outlets of the sample holders. These were connected to a digital differential thermometer (Bailey-Sensortek TH-6D) that allowed a rated precision in the absolute mode of ±0.1°C and of ±0.01°C in the differential mode. The temperature difference was minimized by adjusting the current in the nichrome heater. No temperature increase due to the microwave or electrical heating was detected in the suspensions throughout the experiment. The use of small cuvettes, HELLMA 144 O-NS, and the placement of the head of the peristaltic pump inside the irradiation chamber allowed the use of a total suspension volume of 2.5 ml.

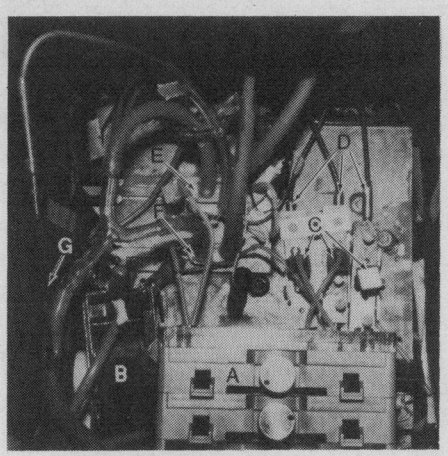

Fig. 4. Top view of the irradiation chamber. The copper tank is at the bottom of the chamber. A metallic lid was placed on the chamber during the experiments. *A*—peristaltic pump; *B*—electric fan; *C*—cuvettes for turbidity measurements; *D*—optical fiber bundles; *E*—sham-irradiation sample holder; *F*—irradiation sample holder; and *G*—temperature probe for water jackets.

D. Optical System

A fiber optic system was used to measure the turbidity of the yeast cultures and in turn the growth rates of the organisms. We used optical fibers made with Crofon 4 (Dupont) fiber bundles (48 fibers, each 0.25 mm in diameter). An interference filter (Edmund Scientific Corporation, 650 nm center wavelength, 10 nm bandwidth) was placed between the optical fibers and a 150 W frosted tungsten lamp. A stabilized ac power supply was used to obtain a light output stable to within ±0.5 percent. The turbidity was determined with the optical fibers placed in contact with the walls of the cuvettes. The incident light intensity at the cuvette was about 6.5 μW, while the transmitted light intensity ranged between 330 and 230 nW. The light intensity was measured with a Photodyne XL88 photometer/radiometer with sensor head model 250.

Results

The possible effects of mm-waves on yeast suspensions were monitored by measuring the growth rate of the cul-

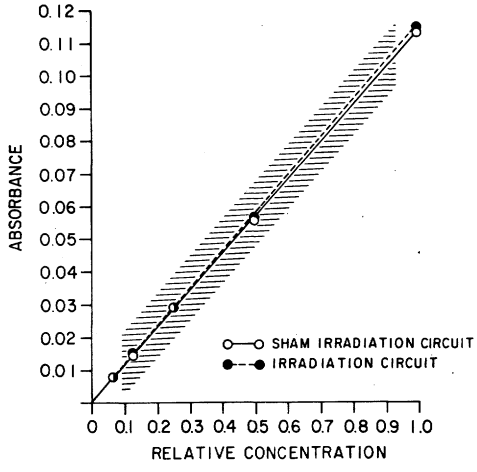

Fig. 5. A typical plot of absorbance versus concentration. A concentration of 1 corresponds to a 1 : 10 dilution of the overnight culture. The points represent the average of three measurements taken at the indicated concentrations. The slope for the "sham-irradiation" circuit had a coefficient of 0.112 with a 95 percent confidence limit of ±0.003. The "irradiation" circuit slope had a coefficient of 0.113 ± 0.002. The lines represent the fitted curves calculated with a linear regression program. The shaded bar indicates the absorbance/concentration range usually used for the experiments.

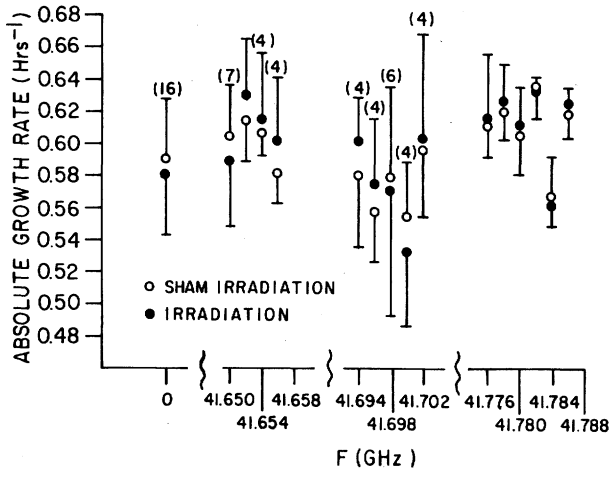

Fig. 6. Effects of millimeter-wave irradiation on the absolute growth rate of *Saccharomyces cerevisiae* as a function of frequency for an absorbed power of 20 ± 0.5 mW. Each point and bar represent the average ±1 SD of the experiments at each frequency. Unless otherwise indicated in parentheses, three experiments were performed at each frequency.

tures and by performing a plate count at the end of each of the experiments. The absorbance was calculated as Abs = log (P_{in}/P_{tr}) where P_{in} was the light intensity transmitted through the reference cuvette filled with Sabouraud glucose broth and P_{tr} was the light intensity transmitted through the cuvettes filled with either the sham-irradiated or the irradiated suspension. Before each experiment, the light transmitted by the three cuvettes, all filled with the growth medium, was verified to be within the resolution of the photometer (i.e., 1 count = 1 nW).

The linearity of the response of the optical system of both circuits was determined before each experiment using a twofold dilution series, whose cell concentration range was close to that of the actual experiment. A typical absorbance versus concentration curve is shown in Fig. 5. For a total of 38 tests, the average percentage difference between the growth rates measured in the two circuits was +0.77 percent, with a standard deviation of 0.57 percent. The maximum difference ever detected was +2.5 percent.

The growth rate was obtained by using a regression analysis to fit the data to an exponential curve $C(t) = C_o e^{\mu t}$, where $C(t)$ is the concentration at time t, C_o is the initial concentration, and μ is the growth rate in h^{-1}. The percentage difference in the growth rate of yeast cells was calculated as $100 \times (\mu_i - \mu_c)/\mu_c$, where μ_i and μ_c are the growth rates for irradiated and sham-irradiated cultures, respectively.

We performed 16 experiments without either mm-wave irradiation or electrical heating, and under such conditions the average difference in growth rates between the "irradiated" and the "sham-irradiated" circuit was −1.5 ± 3.2 percent. The average 95 percent confidence limit of the estimated growth rate was about ±2.3 percent regardless of the circuit or of the presence of mm-waves.

From these data we can infer that, with at least three experiments for each frequency, the minimum difference L, significant at the 95 percent confidence limit, was about ±3.5 percent as calculated from $L = 1.96\ \sigma/\sqrt{n}$, where σ is the average standard deviation, 3.2 percent, and n is the number of experiments [26]. In the three frequency ranges that were covered, the selected frequencies were 2 MHz apart. At least three experiments were performed at each frequency; the actual numbers are indicated in Figs. 6 and 7.

In Fig. 6 the results of mm-wave irradiation on the growth rate of yeast measured optically are shown as averages of the absolute growth rates μ_i and μ_c. In Fig. 7(a) the same data are also plotted as a percentage difference of the growth rates. Paired t tests were performed on the absolute growth rates of irradiated versus sham-irradiated cultures, grouped by frequency. No statistically significant differences were detected ($P > 0.05$) at any of the selected frequencies.

For most of the experiments we also performed a plate count at the end of irradiation. The percentage differences between the number of viable population units of the irradiated culture minus those of the sham-irradiated are shown in Fig. 7(b). No statistically significant differences ($P > 0.05$) were detected by unpaired t test statistics performed for each experiment, except for some experiments at some of the frequencies at which $0.01 < P < 0.05$. This, however, never occurred for more than half of the total number of experiments done at each frequency.

We have also performed some experiments at higher power levels at a fixed frequency of 41.698 GHz. The results are showed in Fig. 8. No effects were detected even at these higher powers.

Discussion

In the past some investigators indicated that the mm-wave [1] at different frequency ranges alters the growth rates of *R. rubra* [1], *Sacch. cerevisiae* [2]–[6], *E. coli*

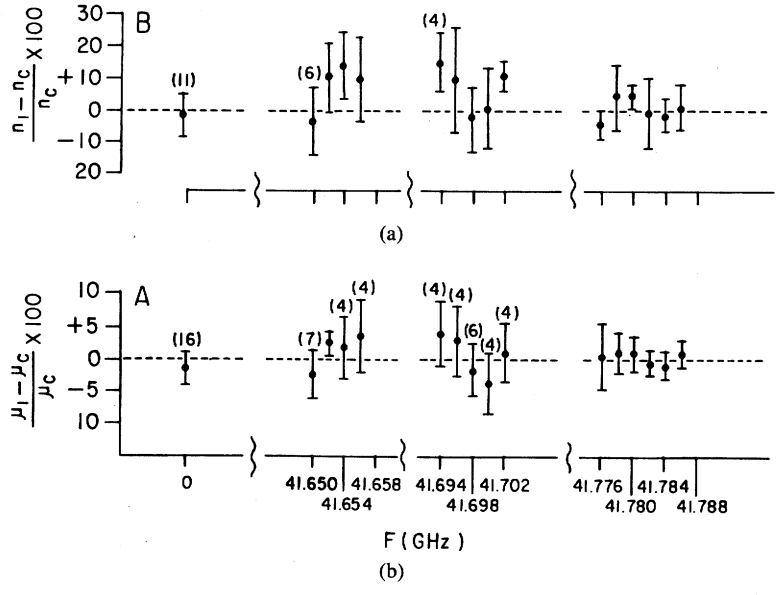

Fig. 7. Effects of millimeter-wave irradiation on *Saccharomyces cerevisiae* as a function of frequency. (a) Percentage differences of the growth rates. (b) Percentage difference in the number of viable populations units. Each point represents the average ±1 SD of the experiments at each frequency. Unless otherwise indicated in parentheses, three experiments were performed at each frequency.

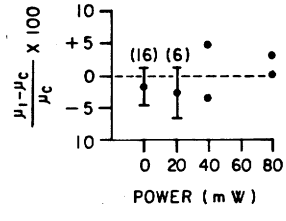

Fig. 8. Effects of higher power levels on the growth rate of yeast. Frequency fixed at 41.6980 GHz. The points at 40 and 80 mW represent the result of single experiments.

[7], and *C. albicans* [9]. Other reports also indicated that mm-waves enhance the induction of colicin [12] and of lambda phage [13] in *E. coli*. These reports prompted several comparative studies on prokaryotic and eukaryotic cells such as *E. coli* [16], *Sacch. cerevisiae* [17], BHK-21 [18], [19], and *S. typhimurium* [22], [23]. All these studies failed to indicate any nonthermal effect of mm-waves. In particular, a study on protein synthesis in mammalian cells BHK-21 exposed at various power levels failed to reveal any change due to mm-waves at 202 frequencies in the 38–48 GHz and 65–75 GHz ranges [19].

As shown in Figs. 6 and 7 the present study supports the conclusions that mm-waves do not affect in a detectable manner the growth rate or the viability of yeast cells. The average growth rate difference of irradiated cultures of *Sacch. cerevisiae* differed by no more than ±4 percent from the sham-irradiated at any of the selected frequencies, while the plate counts did not differ by more than ±15 percent. The larger differences in the plate count may have been due to slight initial concentration differences resulting from sampling errors that might have occurred when the circuits were loaded at the beginning of the experiment. This difference, however, would have not resulted in significant differences in the growth rates.

The spectral characteristics of our microwave source can be favorably compared to those of a similar experiment reported by Grundler *et al.* [6], where the bandwidth was ±0.5 MHz, the frequency stability was ±0.3 MHz, and the absolute frequency was accurate to within ±0.1 MHz. In particular, the use of very accurate frequency counters in both this study and the ones reported in [5], [6] excludes the possibility that we might have used absolute frequencies offest by more than a few hundreds of a kilohertz. Since a systematic scanning of even a small portion of the millimeter-wave band, 30–100 GHz, with a frequency step of 2 MHz would have required an inordinate amount of time, we have focused the experiments at the frequency ranges in which the largest effects have been reported in the past on the growth of *Sacch. cerevisiae* [2]–[6].

The objection could be made that the difference in the results could be ascribed to the differences in strain. This objection postulates the existence of a wild-type strain of yeast that is naturally sensitive to mm-wave radiation. On the basis of experience with induced sensitivity to physical agents, like UV for example, it seems extremely unlikely that this could happen. In the case of UV, for example, the resistance is obtained by selecting those cells (usually 1 in 10^6) that survive UV treatment. In our case the existence of a mm-wave sensitive strain would imply that either a) the strain used by Grundler and Keilmann has been used after becoming sensitive to exposure to mm-wave radiation, or b) we used a strain that for some reason had lost its sensitivity to mm-wave radiation. The probability of either phenomenon happening, even though the

exact probability cannot be determined, seems to be exceedingly small. Furthermore, biological effects of mm-waves have been reported in a variety of different living organisms.

It should be pointed out that the points and the bars shown by Grundler *et al.* in Fig. 9 of [5] do not represent the average and the standard error, respectively, of multiple experiments at the same frequency. Instead, the points are the results of single experiments and the "error bar" was obtained as follows. Two different spectrophotometers monitored the growth of two cultures prepared from the same suspension. The maximum percent difference, over several experiments without microwaves, between the growth rates of the two cultures was then used as the estimated error. Therefore, the "error bars" in Fig. 9 of [5] all duplicate the same information. This representation also implies that the authors of [5], [6] assume the estimated experimental error to remain constant regardless of the irradiation frequency. If all the points within ± 4 percent, i.e., their estimated error, of Fig. 9 in [5] are considered as the experimental "noise," then the use of a three-point interpolated curve to connect all the experimental data with a resonant-like curve becomes quite unjustified. However, even though in [5], [6] there are not enough experimental data at most of the selected frequencies to claim a definite effect on the growth rate, there is an apparent similarity between the results obtained with two independent sets of experiments, using two different irradiation systems. Furthermore, this similarity is also supported by the cross correlation analysis of the two sets of experiments [5], [6].

The experiments reported in [2]–[6] were not done at constant mm-wave power; instead, the power varied from experiment to experiment between 5 and 40 mW, introducing thereby another uncontrolled variable in the experimental procedure. However, both in the present study and in the ones reported earlier [2]–[6], no cell was exposed continuously to mm-wave energy due to the small skin depth of mm-waves and either recirculation or stirring of the cell suspension. In fact, two of the more notable differences between the study in [2]–[6] and the present one are a) the average time for a single exposure (0.06 s versus 2.0 s for the present study); b) the average interval between subsequent exposures (1.2 s versus 30 s). Nevertheless, the total time that the average cell was exposed to mm-wave energy is comparable (5 percent versus 6.7 percent for the current study).

As a suggestion for future studies of biological effects of mm-waves, we would like to point out that in the past when the power used was less than 5 mW/cm^2 such effects have been considered "nonthermal" in nature [2]–[8], [13], [17], suggesting that the microwave-induced heating could not have caused the reported effect. However, the skin depth δ is very small in lossy materials such as biological media containing large amounts of water. For example in distilled water, δ is between 0.78 and 0.23 mm for frequencies in the range 30–300 GHz. This leads to very high values of the specific absorption rate (SAR) in the surface layers of the exposed medium even for apparently small incident power densities. For example, at 1 mW/cm^2, the surface SAR in water at 40 GHz is 18.4 W/kg. On the other hand, the power density P decreases exponentially as $P = P_i e^{-2x/\delta}$ where P_i is the incident power density and x is the distance from the surface exposed to the incident field. Therefore, any organism located more than 3–4 skin depths from the exposed surface is subjected only to a negligible fraction of the incident mm-wave radiation.

It is therefore important to determine, on an individual basis, whether such high values of power deposition can lead to subtle thermal effects or not. Some of the reported effects have been more appropriately called nonthermal when either of the following circumstancs occurred: a) the microwave irradiation induced an effect opposite to that expected by a comparable increase in temperature; b) when the effect appeared to be strongly dependent on the irradiation frequency.

Conclusions

The current experiments were specifically designed to gather data that would help establish the presence or absence of nonthermal effects and frequency specific effects of mm-waves on the growth of cells. This effect, first reported in 1968 [7] for the growth of *E. coli* was followed by other reports indicating specific induction of lysogenic *E. coli* [13], colicin induction [12], and others [1]–[6], [8], [9]. The fundamental and empirical significance of these alleged frequency specific actions of nonionizing radiation in the mm-wave range hardly needs any emphasis.

Contrary to the previous reports [2]–[6] from other investigators, millimeter waves did not induce any detectable effect on either the growth rate or the viability of yeast cells exposed for 4 h to ultrastable millimeter waves between 41.650 and 41.798 GHz. This report adds to a growing list of others [15]–[23] showing that under carefully controlled experimental conditions, no nonthermal effects of millimeter waves on unicellular organisms are evident.

This conclusion suggests extreme caution when unconfirmed reports are used as a basis for broad generalizations of biological effects of millimeter waves [24], [25] by authors who also suggest the possible use of mm-waves for cancer diagnosis [27], [28] and therapy [29], and a possible means of cell communication [24].

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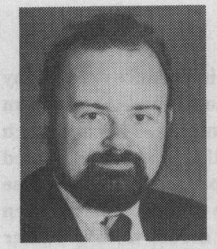

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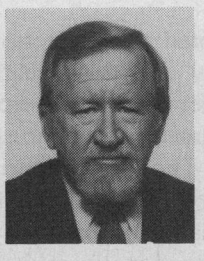

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