Resonant Microwave Effect on Locally Fixed Yeast Microcolonies

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The microwave influence on the growth of yeast cells is studied in a novel experimental set-up designed to observe individual cells growing for several division cycles. The results are in accordance with resonant microwave-induced growth stimulation as observed in our earlier set-up where the turbidity of a stirred suspension of cells was used as the measure of growth. The new experimental set-up is suited to decide on the proposed triplet mechanism of resonant microwave biological effects.

Introduction

Research on biological effects of microwaves is characterized by a large number of open questions. While many authors have reported certain effects to exist, repeats of their studies by others led to an opposite conclusion in several cases. Most studies, however, were not repeated at all. Only in a limited number of cases agreement on the existence of the effects has been reached. Not surprisingly, this happened for just those cases where a theoretical model had been put forward, as for example in case of the microwave auditory effect.

The reason for the unsatisfactory state of the field may well lie in the complexity of either the biological processes or of the microwave interaction. The reported microwave effects are subtle, indeed often at the limit of observability, and then of course do not easily allow studies of specific influences of *e.g.* power or frequency. The yeast growth experiments reported here are quite an exception since even though the microwave effects are small the continuity of the laboratory work has allowed a repeated establishment of the resonant feature of this effect [1].

In this contribution we demonstrate both a novel way of observing the growth of yeast cells and a confirmation of the resonant microwave effect, near 42 GHz, even under the new experimental conditions. The apparatus is built to observe the growth of cells fixed in space. The well-separated cells can be subjected to identical microwave exposure which is constant in time. Thus the set-up can serve to investigate specific influences, in a single run, *e.g.* of either microwave power or microwave polarization. Specifically, the experiment should be able to decide on the prediction of a recent model which bases the resonant effect on the interaction of microwaves with molecular spins *via* the magnetic field component of the microwave [2].

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Materials and Methods

Microwave growth experiment

Yeast cells are fixed to the lower surface of a thin transparent agar layer. Each cell develops into a microcolony of eight cells. The agar layer rests on a sapphire plate mounted in a temperature-stabilized chamber. The chamber is scanned under a microscope for automatic observation of the cells. To apply the radiation microwaves from a stabilized source as used earlier [1, 3] are guided through the sapphire plate on which the cells are spread in a monolayer. An automatic microphotographic method is used to continuously record the development of about 50 microcolonies [3].

Materials and preparation

Diploid yeast cells *(Saccharomyces cerevisiae)* were obtained from a stock culture of stationary state

cells on solid nutrient agar (20 g/l glucose, 20 g/l Bacto agar, 5 g/l yeast extract; 4 °C) and suspended in phosphate buffer. A synchronized subpopulation (early G_1-phase) was selected by volume sedimentation within a linear density gradient. These cells are characterized by a mean volume of 30 μm^3, a mean duration of the first G_1-phase of about 3 h and a mean cell cycle time of 1.35 ± 0.03 h. For the microscopic observation 10 µl of a buffer suspension containing $4 \cdot 10^6$ cells/ml are filled into the cell chamber shown in Fig. 1.

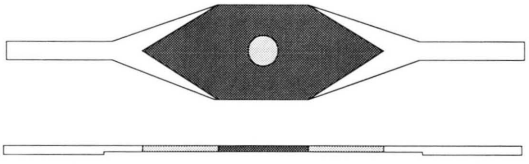

Fig. 1. Chamber for microscopic observation of growing cells located on an oversized dielectric wave-guide.

The microscope slide is a sapphire plate, chosen because of its high thermal conductivity. Two concentric plastic rings with thicknesses of 0.1 mm and 0.2 mm, for the inner and outer ring, respectively, are fixed on the plate. The inner ring is filled with warm nutrient agar, a cover slide (0.5 mm thick) is put on top, and the sample is stored at 4 °C for several days. Then the cover slide is removed with the agar adhering to it, and 10 µl of cell suspension containing approximately 10^4 to 10^5 cells are put in the inner ring. Finally the cover slide with the agar is put back and fixed in position by applying vaseline on the outside.

The chamber is well suited for observation of cell growth. The cells lie in a well-defined plane. They are surrounded by a thin fluid film, and the slight pressure from the agar layer prevents them from migrating. Further the newborn cells arrange in a monocellular layer. This is of value not only for the microscopic observability, but also provides good thermal contact to the sapphire plate and allows homogeneous irradiation conditions.

Microwave irradiation

The sapphire plate is part of the microwave circuit, in fact it is the dielectric in a dielectric-filled metal wave-guide. This wave-guide section is oversized to a width of 30 mm, in the dimension perpendicular to the microscope view direction. The sapphire plate is mostly covered with gold to confine the microwaves, except at the ends (where the microwave is coupled in and out to standard wave-guide by proper tapers), and within a circle of 10 mm in diameter where the cells are placed. A similar opening is provided at the opposite surface to allow microscope viewing, but this is covered with a fine metallic mesh structure which completely suppresses leakage of microwaves to that side.

While in this set-up the microwave field in the sapphire can be expected to be multimode and thus difficult to characterize, the transmitted power P_t to the output wave-guide can be measured. With an input power of 20 mW we find P_t to be 0.12 mW, somewhat dependent on frequency (± 20% in the 40 MHz range of Fig. 3b). This value however doubles when the sample (*i.e.* glass slide, agar and cells) is replaced by a metal plate from which we estimate that the sample cells are exposed to a microwave intensity of the order of 1 mW/cm^2.

The microwave-induced heating of the sample can be estimated to be fully negligible because of the presence of the sapphire plate. The sample chamber is temperature-stabilized using a water bath to 31 ± 0.1 °C.

Observation of growth

The bright-field microscope image of the cells is automatically recorded on film every 5 min and subsequently digitized by a TV camera. This is done for six different positions on the sample within a frame of $0.54 \cdot 0.36$ mm^2. Altogether about 50 cell colonies are observed simultaneously. Since the cells appear as white rings on a dark background, approximately 6 µm in diameter, they can well be discerned by pattern-recognizing programs. The appearance of budding can be determined with high resolution. This budding coincides with the transition of the cell from the G_1 to the S phase. Therefore, when we define the cell cycle time by the time between consecutive budding our method allows us to determine the cell cycle for each of the cells individually.

The evaluation of experimental data thus yields the distribution of cell cycle times, either separately for the first generation (= first-to-second budding), for the second generation or for all generations together.

Results

We conducted a series of 42 growth experiments, 25 with and 17 without microwave irradiation. For the former, the input power ranged from 13 to 23 mW. Fig. 2 gives several examples of the resulting distribution of cell cycle times. In these runs the control experiments, *i.e.* those with no microwaves present, resulted in the narrowest distributions. The effect of irradiation can be seen in Fig. 2 to be an

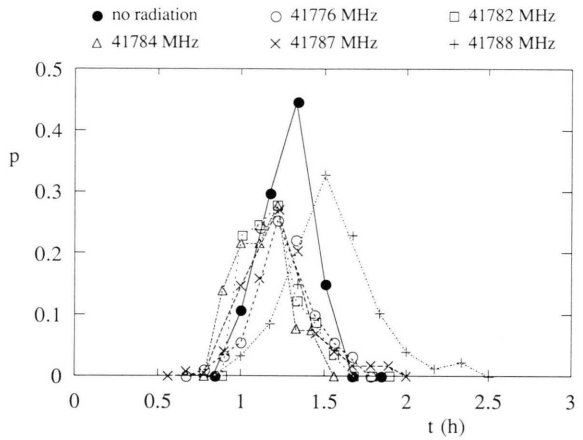

Fig. 2. Distribution p of cell cycle times t for the first two generations, normalized to $\int p dt = 1$. The lines are only drawn to guide the eye.

asymmetric deformation of the distribution. Any broadening must mean that not all cells respond in the same way. For further evaluations we disregard this effect and concentrate on the shift of the distribution, by calculating the average cycle time t_{av} in each distribution. A statistical analysis was employed to find out whether t_{av} was significantly changed by the irradiation; the result is positive, on a highly significant level > 0.99 (student t-test), for all five distributions in Fig. 2. By taking the inverse of t_{av} we obtain the average growth rate t_{av}^{-1}, which we further normalize to the mean value obtained in the control runs. Fig. 3 shows the resulting normalized growth rate μ for different exposure conditions.

Discussion

The data obtained from the new experimental set-up prove again the existence of a microwave effect on the growth on yeast cells. Specifically, while we observe now that for the individual cell the effect can be a shortened or prolonged duration of the cycle, we find that for an ensemble of cells located in close vicinity the average effect does not vanish but lies in the order of magnitude of the effect established earlier with the photometric measurement of stirred cell suspensions.

The agreement between the earlier and the present experiments is further supported by the frequency dependence. The present experiment yields a resonance of growth enhancement centered near 41,782 MHz with a width of about 8 MHz, in close accordance to what was observed with the photometer. Even the slight negative effect at the foot of the resonance profile seems to be reproduced. We therefore conclude that the new set-up fully substantiates our former claim of a resonant and non-thermal effect.

The aspect of a microwave-induced temperature rise can be specifically discussed for our new set-up. The cells are embedded in an aqueous surrounding. We assume like before that the dominant microwave absorption mechanism – even in the cells – is the relaxational absorption of water [1]. Therefore the cells are in thermal equilibrium with the surrounding

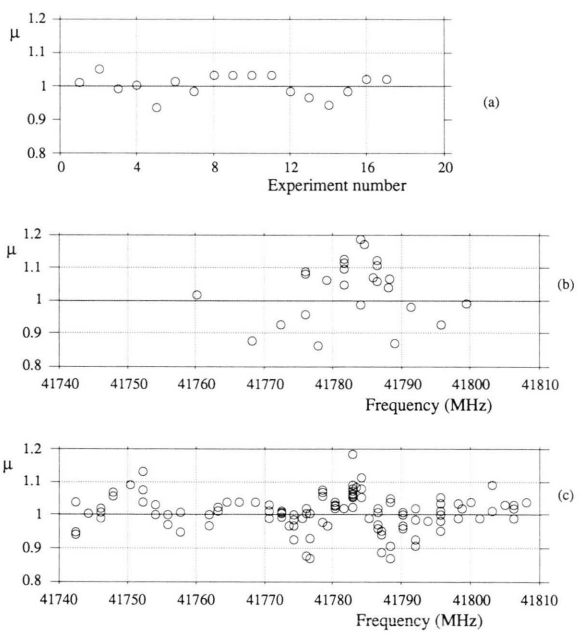

Fig. 3. Normalized growth rate of population obtained by averaging over individually determined cell cycle times; (a) without and (b) with microwaves near 42 GHz. For comparison with earlier results, the lower graph (c) gives the microwave effect on aqueous yeast suspensions determined by turbidity [1].

water. The layer consisting of agar and cells is heated by the microwaves and cooled by thermal conduction into the sapphire substrate. For a simple estimate the heating can be assumed to be homogeneous for a layer thickness of $d = 200$ μm since this is the microwave absorption length in water. For cooling we consider one-dimensional heat conduction to find $\Delta T = d \cdot L/2\lambda$, where $L \leq 1$ mW/cm^2 is the absorbed power density in the experiments and $\lambda = 0.006$ Wcm^{-1}C^{-1} is the heat conductivity of water. We obtain a steady-state temperature difference of at most 0.002 C at the distance of 200 μm from the sapphire; the value at the location of the cells, near to the sapphire surface, can be estimated to be even smaller by an order of magnitude. Because of the smallness of the microwave-induced heating the observed effect is not likely to be a consequence of heating, *e.g.* it is non-thermal.

Conclusion

The resonant non-thermal microwave effect on yeast growth has been confirmed. The effect has not changed when the growth environment was changed from a stirred liquid suspension to a stable agar surface.

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