

Activation of Metabolism of Nonphotosynthesizing Microorganisms with Monochromatic Visible (Laser) Light: A Critical Review

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The light-growth responses of *Escherichia coli* WP2 tip⁻ and yeasts *Torulopsis sphearica*, *Saccharomyces ludwigii*, *Saccharomyces cerevisiae* 14, *Candida mallosa*, *Candida boidinii*, and *Endomyces magnusii* have been reviewed. The photosensitivity of these nonphotosynthesizing microorganisms to monochromatic bands of visible light depends on light parameters (wavelength, intensity, dose, pulse repetition rate) as well as on metabolic state of the cells. Irradiation with a He-Ne laser ($\lambda = 632.8$ nm) was also found to increase the number of germinated and outgrown endospores of *Anaerobacter polyendosporus*.

KEYWORDS: *endospores, germination, light-growth response, low-power laser effects, nonphotosynthesizing microorganisms, vegetative cells, yeasts*

1. INTRODUCTION

Light, one of the important environmental factors, plays a critical role in photosynthesis. The nonphotosynthetic action of light is also well known; processes like vision, phototropism, phototaxis, photomorphogenesis, photobiosynthesis of carotenoids and vitamin D have been the subjects of investigations for decades.

There exists only a small body of data about light-growth responses of nonphotosynthesizing microorganisms (reviews: Carlile, 1965, 1970; Rubin *et al.*, 1971; Erianger, 1976). In most cases the inhibitive effects of visible, especially blue, and near UV light on growth and division of both eukaryotic and prokaryotic microorganisms were studied (Epel, 1965; Ehrenberg, 1966; Krinsky, 1976; Webb, 1977; D'Aoust *et al.*, 1980; Jagger, 1981; Senger 1982; Peak *et al.*, 1983; Macmillan *et al.*, 1966; Webb and Brown, 1976; Edmunds, 1980; Ninneman *et al.*, 1970). There is much less information about stimulation of vegetative growth of microorganisms by various wavelengths of visible light. The nonphotosynthesizing microorganisms studied with the aim of stimulating their metabolism with monochromatic visible (laser) light were the following: *Pseudomonas fluorescens* (Greppin and Gouda, 1965a, b), *Blastocladiella emersonii* (Cantino and Horenstein, 1956); *Candida guilliermondii* (Fraikin *et al.*, 1973); *Lactobacterium delbrukii*,

Clostridium butyricum, *Bacillus subtilis*, *Bacillus brevis*, *Pseudomonas fluorescens*, *Schizosaccharomyces pombe*, *Saccharomyces vini*, *Aspergillus oryzae* (Fraikin *et al.*, 1974); *Torula utilis* (Konev *et al.*, 1970); *Propionibacterium acnes* (Kjeldstad, 1987); *Paramecium* (Hutchinson and Ashton, 1929); *Torulopsis sphaerica* (Chebotaryov and Zemlyanukhin, 1970); *Rhodotorula gracilis* K-1 (Kovalchuk *et al.*, 1982); *Saccharomyces cerevisiae* 14 (Abramova, 1978); *Cunninghamella japonica* (Poletebnova *et al.*, 1982); *Escherichia coli* K-12 mutants AB1157 and Gam 444 (Voskanyan *et al.*, 1985,1986); seven strains of *E. coli* derived from K-12 (Bertoloni *et al.*, 1993); *E. coli* B (Daniels and Quickenden, 1994; Quickenden *et al.*, 1994); *Saccharomyces cerevisiae* (Quickenden and Daniels, 1993). It was found in most of these studies that division rate, duration of growth phases, and total yield of biomass can be influenced by the irradiation. In the paper of Quickenden *et al.* (1995), an increase in intracellular pH was measured after irradiation of *E. coli* with an HeNe laser. We earlier suggested that the pH jump due to irradiation was a crucial step in metabolic rearrangement after irradiation (Karu, 1988; Tiphlova and Karu, 1991b).

It is noteworthy that even in the case of photosynthesizing microorganisms the regulatory nature of visible (blue) light on metabolism was found to be radically independent of photosynthesis (Voskresenskaya, 1972).

Our photobiological interest in light-growth responses of microorganisms was derived from a developing role of laser phototherapy (also termed as laser biostimulation) in treating patients with various local and systemic diseases (reviews: Karu, 1987,1989a, b). The method described in the present paper was developed for studying the molecular mechanism of laser therapy. In the beginning of the 1980s, there was a certain disparity between the active work of physicians in this field and the lack of interest shown by physicists, chemists and biologists. Nowadays, most of the dubious points of the laser biostimulation (low doses and intensities of the light, possible role of light coherence, versatility of the effects, various magnitudes of the effects and sometimes lack of any effect) have been discussed and explained (Karu, 1987, 1989a, b, c). During the past few years, numerous reviews on the topic of low-power laser effects have been written (Karu, 1987,1988,1990,1992,1996; Belkin *et al.*, 1988; Harris, 1988; Bems *et al.*, 1988; Basford, 1989; Smith, 1991; Tiphlova and Karu, 1991) describing various aspects of the problem: history (Karu, 1987, 1989b), controversies and limitations (Karu, 1987, 1989c, 1996; Basford, 1984; Belkin *et al.*, 1988), quantitative laws of visible light action on cells (Karu, 1987, 1989b, 1996), molecular mechanism (Karu, 1989b, 1988,1996; Tiphlova and Karu, 1991b). Specific data about the effects of irradiation on various cells has also been reviewed, including: mammalian cells cultured *in vitro* (Karu, 1990), *Escherichia coli* (Tiphlova and Karu, 1991b), and human lymphocytes (Karu, 1992). The aim of the present paper is to describe in detail the method of growth stimulation of microorganisms by light. Also the difficulties and possible controversial points of the method will be discussed. This method may be of interest not only in laser medicine but also in photobiology and microbiology, especially as regards the metabolism regulation mechanisms.

2. MATERIALS AND METHODS

2.1. Microorganisms and Their Growth

I.I.I. Escherichia coli. The wild-type of *Escherichia coli* WP2 *trp*⁻ was obtained at the Genetic Faculty of the Moscow Lomonosov University. The culture was grown in Hottinguer broth (unless noted otherwise), pH = 7.0 (20 ml of culture inoculated 1:20) at 37°C

during one night in a 50-ml Erlenmeyer flask without stirring. Once concentration of $(1 \text{ to } 2) \times 10^8$ cells/ml was reached, the cells were harvested on a nitrocellulose filter (Millipore 0.4 μm), washed twice with a 0.05 M K, Naphosphate buffer, pH = 7.0, and resuspended in the same buffer to a concentration of $(1 \text{ to } 2) \times 10^9$ cells/ml. This suspension was used to the irradiation (Section 2.2).

2.1.2. *Yeasts*. The yeast-like fungus *Endomyces magnusii* reproducing by division and yeasts *Torulopsis sphaerica*, *Saccharomyces ludwigii*, *Candida boidinii*, *Candida maltosa*, *Saccharomyces cerevisiae* 14 reproducing by budding, were studied. The microorganisms were obtained from V.K.M. (All-Union Collection of Microorganisms, Moscow). The yeast cultures studied differed substantially by size of individual cells, structural organization, activity of respiration and fermentational processes, reproduction rate, and sensitivity to various chemical and physical agents. Some of the biochemical characteristics of the yeasts used in our experiments are presented in Table I.

After growing for 24 h on wort-agar slants, the cells were transferred to rocker-mounted flasks with liquid nutritive medium (wort 7° Balg or Reder medium with 1% glucose or 1% methanol added as a source of carbon). The cells were grown in liquid nutritive medium at 28°C on shakers with intensive aeration for 5 to 24 h to obtain the required number of cells at the logarithmic growth phase (Fedoseyeva *et al.*, 1988a, b). We also tried to grow the yeast in Petri dishes in agar-wort, but this method did not, except in case of *T. sphaerica* (Fedoseyeva *et al.*, 1984), yield the required number of cells. The yeasts were separated from the nutritive medium by centrifugation and washed with twice sterile tap water. The suspension with optical density 0.06 ($\lambda = 540 \text{ nm}$, optical path 0.3 cm) in K,Naphosphate buffer (pH = 6.0) was prepared for the irradiation (Section 2.2).

2.1.3. *Anaerobacter polyendosporus*. *Anaerobacter polyendosporus* (*Bacillus*), obtained from Prof. V. I. Duda, Institute of Microbiology of the Russian Academy of Sciences, is a Gram positive facultative anaerobic strain separated from soil of Birman rice fields (Duda *et al.*, 1985). By sporulation, one cell of this strain forms from 3 to 5 endospores. The culture was grown on a mineral-agar medium with a composition according to Pfenning and Lippert as described in (Kutomkina *et al.*, 1991). The cells were allowed to grow and sporulate for 7-9 days in aerobic conditions at 28°C until practically all spores had been released from the sporangia. The spores were collected on Watman N=1 filter paper, washed

TABLE I
Biochemical characteristics of the yeast cultures under study

Culture	Respiration activity in QO_2 ($\mu\text{l/mg}\cdot\text{h}$)	Overall content of flavins ($\mu\text{g/Dig protein}$)	Content of flavin mononucleotides ($\mu\text{g/mg protein}$)	Catalase units of special activity
<i>Saccharomyces ludwigii</i> (glucose)	-76.9 ± 6.9	23 ± 3	22.0 ± 0.8	0.47 ± 0.03
<i>Candida boidinii</i> (glucose)	-77.7 ± 2.1	33 ± 2	23.1 ± 0.3	1.70 ± 0.05
<i>Torulopsis sphaerica</i> (glucose)	-117.6 ± 6.7	21 ± 4	18.9 ± 0.6	6.18 ± 0.02
<i>Candida boidinii</i>	—	36 ± 2	31.1 ± 0.2	16.3 ± 0.01
<i>Candida maltosa</i> (glucose)	-61.0 ± 2.0	110 ± 6	63.7 ± 0.5	11.8 ± 0.1
<i>Saccharomyces cerevisiae</i> (glucose)	-50 ± 5.8	34 ± 4	17.0 ± 0.6	1.82 ± 0.05

Experimental details can be found in Fedoseyeva *et al.*, 1988a.

repeatedly with sterile distilled water, and a suspension in a concentration of 4×10^8 cells/ml in the distilled water was prepared for the irradiation (Section 2.2).

2.2. Irradiation

2.2.1. Light sources. Both conventional light sources (lamps coupled with appropriate glass and/or interference filters or with a monochromator) and lasers can be used inasmuch as the "laser biostimulation" is based on photochemical conversion of photoabsorbing molecules in a cell (Karu, 1987, 1989a, b, c; Smith, 1991). In other words, the laser biostimulation is a photobiological method and such a specific characteristic of the light like coherence is not needed (Karu, 1987; Smith, 1991). Laser sources are just handy tools providing many practical advantages (e.g., efficient fiber optic coupling, high monochromaticity and easy wavelength tunability, simplicity in use and electrical safety in the case of semiconductor lasers). In deciding on a particular light source, it is important to use an appropriate wavelength (dependences on light wavelength are discussed in Section 4.3) with the monochromaticity within the bandwidth of absorption spectra of photoacceptor molecules.

Glass filters or monochromators coupled with conventional light sources were used for recording the action spectra (Fedoseyeva *et al.*, 1988a; Karu *et al.*, 1983; Tiphlova and Karu, 1988). In most studies the following laser sources were used: He-Ne laser ($\lambda = 632.8$ nm) (Fedoseyeva *et al.*, 1984, 1988a, b; Kutomkina *et al.*, 1991; Tiphlova and Karu, 1988, 1991a, b; Karu *et al.*, 1991, 1993a, b), Ar⁺ laser ($\lambda = 454$ nm) (Tiphlova and Karu, 1988), semiconductor lasers emitting various red and near infrared (IR) wavelengths (Karu *et al.*, 1990, 1994; Zharov *et al.*, 1987). The desired light parameters such the intensity and dose depend on the object of irradiation as well as on irradiation procedure and will be considered in Sections 3 and 4.

2.2.2. Irradiation procedure. Irradiation was performed in dark or in dim natural light at room temperature without thermostatic control and stirring. Inasmuch as the irradiation lasted for a short period (from some tens of second to some minutes), neither thermostatic control nor stirring were needed (Fedoseyeva *et al.*, 1988a; Kutomkina *et al.*, 1991; Tiphlova and Karu, 1988, 1991a; Karu *et al.*, 1983). Stirring appeared to be damaging even in case of budding yeasts (Fedoseyeva *et al.*, unpublished).

Extraneous illumination (sunshine or artificial light, especially that from the fluorescent bulbs) was carefully avoided during preparation of the suspension and the irradiation.

The suspension of the microorganisms under irradiation filled the following requirements: small thickness of the layer (not more than several millimeters) and proper optical density. In our experimental conditions, the optimal optical density of yeast suspension was found to be 0.06 (Fedoseyeva *et al.*, 1988a). At higher optical densities of suspension, an increased light scattering as well as partial screening of cells occurred. At densities 0.10-0.15, it was practically impossible to perform the irradiation. Table II presents some examples of the relationship between optical density of suspension and required irradiation doses when the yeasts were irradiated with a He-Ne laser.

In case of *E. coli*, the suspension containing $(1 \text{ to } 2) \times 10^9$ cells/ml was irradiated. This density was found to be optimal in our irradiation conditions (Karu *et al.*, 1983).

In case of *A. polyendosporus*, suspension with density of 4×10^8 cells/ml was irradiated (Kutomkina *et al.*, 1991).

Figure 1 presents three principal irradiation schemes used in our experiments. In most cases, the irradiation was performed in glass or quartz flasks with diameter of 24 mm (layer of the suspension was 2.2 mm) or 75 mm (layer 1.1 mm in this case) from the bottom of the

TABLE II
Relationship between optical density of yeast
suspension and optimal dose ($\lambda = 632.8$ nm) for
protein synthesis stimulation

Yeast	Suspension density	Optimal dose,
<i>Torulopsis sphaerica</i>	0.035	340
	0.060	460
	0.135	560
<i>Saccharomyces</i>	0.030	23
	0.051	31
<i>Candida boidinii</i>	0.035	152
	0.060	300

Experimental details can be found in Fedoseyeva *et al.*, 1988a.

flask (Fig. 1A). The flask with the diameter of 24 mm was used in case of *E. coli* and the flask with the diameter of 75 mm, for irradiation of yeasts and *A. polyendosporus*. The beam was expanded with a lens (flask with the diameter of 24 mm) or a special objective (flask with the diameter of 75 mm) to provide an uniform illumination of whole area (Fedoseyeva *et al.*, 1988a, b, 1983; Kutomkina *et al.*, 1991; Tiphlova and Karu, 1988, 1989, 1991a; Karu *et al.*, 1993a, b, 1983). Irradiation from the bottom (Fig. 1A) was preferred to avoid the "meniscus effect" which occurs when irradiating from above. In this case, the meniscus of the liquid acts like a lens and the dose the light reaching the cells (if reaching the lower layers at all) will be decreased in an uncontrolled way. In these cases when the irradiation should be performed from above (e.g., for some technical reasons), one can recommend the scheme in Figure 1C that we used for irradiation of mammalian cells suspension. Here, specially designed flasks fully filled with the suspensions were used.

In some types of experiments, a quartz cuvette presented in Figure 1B was used (Karu *et al.*, 1991, 1994). This type of cuvette allowed irradiation of only a small number of cells, but provided the possibility of using high intensities of nonfocused laser beam. This cuvette can be easily temperature controlled if needed. However, the most recommended irradiation scheme is shown in Figure 1A, which also allows irradiation of an appropriate number of cells.

2.3. Evaluation of the Irradiation Effects

The irradiation effect, growth stimulation, was evaluated by counting the number of viable cells (in case of *E. coli* details will be described in Section 3.1 and case of yeasts, in Section 3.2), buds (Section 3.2), as well as by measuring the amount of synthesized protein (Section 3.2). These were the basic measurements performed to characterize the quantitative laws of light action on the growth of microorganisms (dependences on wavelength, dose, intensity, pulse repetition rate) upon metabolism of prokaryotic and eukaryotic microorganisms. These measurements will be described in Sections 3 and 4.

The data about modification of light-growth effects with chemicals (Karu, 1989b; Tiphlova and Karu, 1991b; Karu *et al.*, 1993b), dependence of these effects on growth conditions (Tiphlova and Karu, 1991a; Karu *et al.*, 1993a), changes in oxidative and phosphorous metabolism in irradiated cells (Fedoseyeva *et al.*, 1988b), modification of *E. coli*-bacteriophage T4 interaction (Tiphlova and Karu, 1989), proposed molecular mechanism of light effects (Karu, 1989b, 1988, 1995; Tiphlova and Karu, 1991b; Karu *et al.*, 1991) can be found in the respective references.

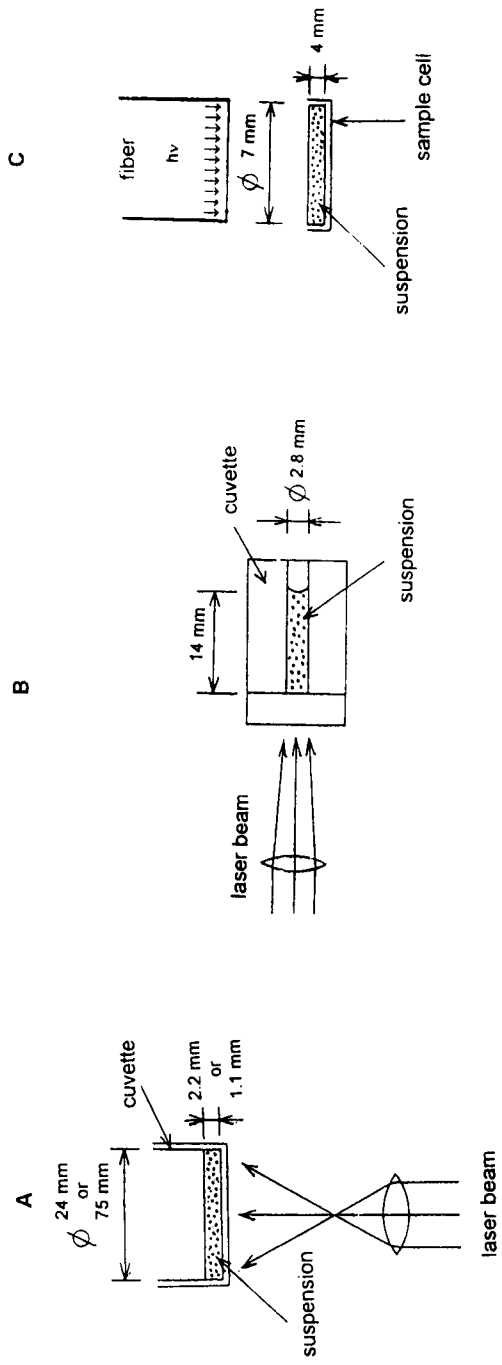


FIGURE 1 Principal schemes of irradiation procedure.

3. LIGHT-GROWTH RESPONSES

3.1. Growth Stimulation of *Escherichia coli*

Figure 2 presents control growth curves of *E. coli* WP2 *trp*⁻ and the growth curves after irradiation the cells with He-Ne laser and inoculation into different nutritive media (Karu *et al.*, 1983; Tiphlova and Karu, 1988, 1991a). Recall that during the irradiation, the cells were in the buffer and did not divide (Sections 2.1 and 2.2). After the incubation in the nutritive medium for time interval shown in the abscissa, the number of colony forming cells per milliliter was assayed by the standard surface plating technique (Tiphlova and Karu, 1988). It is important to emphasize that for every data point presented in this figure, its own portion of cells was irradiated and assayed.

The effect of the irradiation manifested itself in the reduction of the latent period (Fig. 2A). The sharp increase in the number of cells during the first 45 to 60 min of incubation of the irradiated culture (curve 2) as against the weak growth of the unexposed culture (curve 1) corresponds to a 160% stimulation effect. Thus, one of the factors determining the level of stimulation is the time elapsed after the inoculation of irradiated in the buffer solution bacteria into the nutritive medium. The term "growth stimulation" in the text below means the difference between the viable cells in the irradiated culture and the control culture measured by the surface plating technique after 60-min incubation with a nutritive medium.

The growth curves of nonirradiated cells vary depending on the growth conditions. In Figure 2A, the Hottinguer broth was as the nutritive medium (Tiphlova and Karu, 1988). Figure 2B-D depicts the growth curves when the M9 minimal growth medium supplemented with glucose, glycerol, or arabinose was used (Tiphlova and Karu, 1991a). A comparison of the curves 1 and 2 in Figure 2A-D makes it possible to reveal the characteristic features of influence of irradiation with red light on the growth off. *coli* WP2 *trp*⁻ cultures. The difference between the number of cells in exposed and unexposed cultures is small for cultures with a short latent period (e.g., Fig. 2B, C), and large when the control culture begins to divide after a long lag-period (e.g., Fig. 2A, D). So, the magnitude of photostimulation of *E. coli* division depends on the metabolic state of the initial culture or, in other words, on the presence and duration of the latent period. The effect shows up just for a short time, being maximal during 45-60 min after the beginning of the incubation. On the other side, there exists a maximal specific rate of exponential growth (in our experimental conditions, 0.80 h⁻¹) which limits the magnitude of the photostimulation effect (Tiphlova and Karu, 1991a).

The transient acceleration of cell division was found to be especially evident in the case of some mutant *E. coli* strains (Table III). Besides, the cells in the stationary phase of growth were much less photosensitive than the same cells in the logarithmic phase of growth (Bertoloni *et al.*, 1993). The most photosensitive strain from those cited in Table III appeared to be AB1157. It was found also that a radiomodifying effect of He-Ne laser radiation (*E. coli* strains AB1157 and Gam 444 were irradiated before or after exposure of the cells to α -particles or γ -radiation) on *E. coli* K-12 strains was genotype-dependent (Voskanyan *et al.*, 1985, 1986).

Morphological studies showed that the irradiation with red light at 633 nm increased packing of the cytoplasmic matrix and number of ribosomes and decreased until almost complete disappearance of apical vacuoles. Electrophoretic changes did not involve proteins from outer membrane but the protein bands from the cytoplasmic membrane were significantly intensified (Bertoloni *et al.*, 1993).

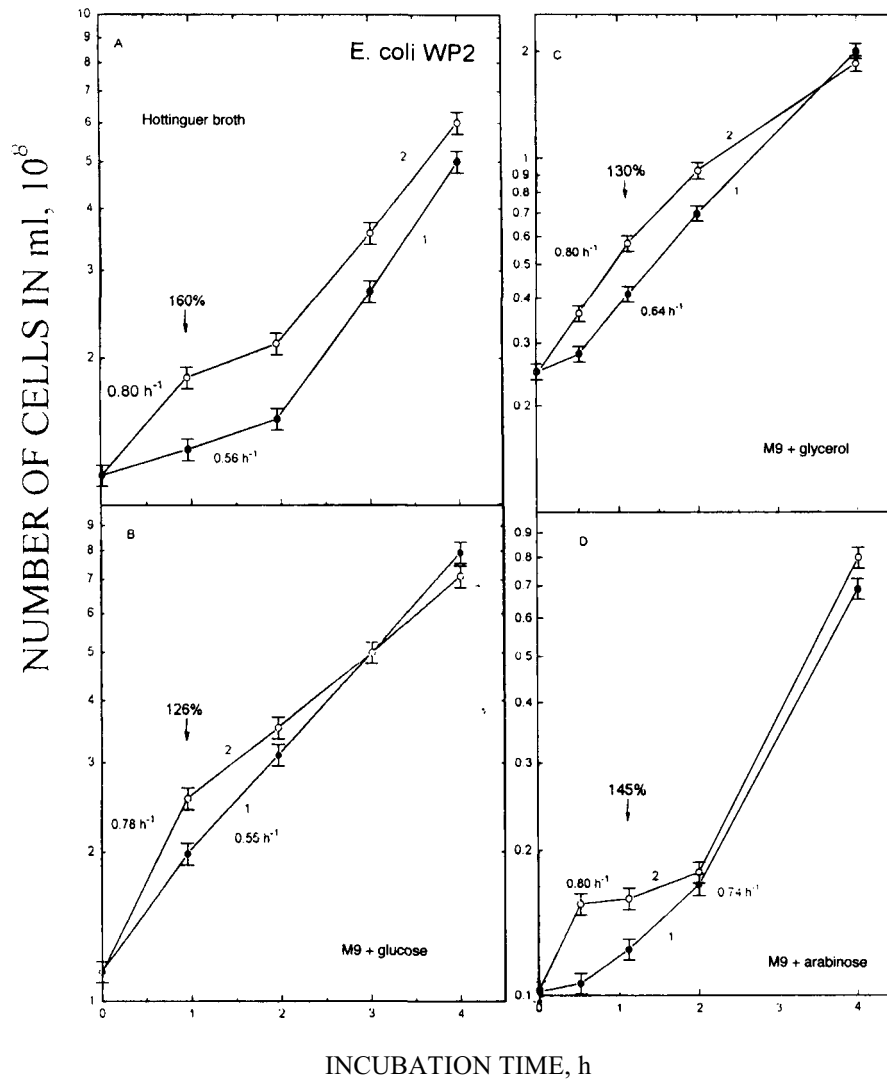


FIGURE 2 Growth curves of (1) control and (2) irradiated with He-Ne laser cultures of *E. coli* WP2 ($D = 4 \times 10^3 \text{ J/m}^2$) cultivated in (A) Hottinguer broth or in M9 medium supplemented with (B) glucose, (C) glycerol, or (D) L-arabinose. The experimental details are described in Tiphlova and Kan, 1988, 1991a.

TABLE III

E. coli strains derived from K12 which photosensitivity was investigated in Bertoloni *et al.*, 1993

No. Strains	Genotypes
1 db1344	araD, argF-lac, flb, pts, relA, rpsL, lamB(am), deoC
2 921	thr, leu, met, las, nal, tonA, hsdR
3 J53	pro, met
4 AB1157	thr, leu, pro, arg, his, thi, lac, gal, rpsI, supE
5 db1229	his, trp, las, Sm, Tn10
6 db1245	Sm, ura, arg, thi, his, ade, lac
7 CGSC6405	araD, argF-lac, flb, non-9, gyrA, relA, rpsL, metE, btu::Tn10, thi, deoC

The results of the experiments with *E. coli* WP2 batches with various initial parameters (Fig. 2) show that only a certain slow-growing subpopulation may react to the photo-stimulus. It was proposed that on the membrane of such a cell, the pH gradient necessary for cell cycle initiation has been achieved (Tiphlova and Karu, 1991b). A scheme explaining the growth curves of control and irradiated *E. coli* cultures is presented in Figure 3. The growth curves of both control and irradiated cultures are supposed to reflect the relation between pH, size of membrane area, and quantity of H⁺ transport enzymes in light sensitive bacterial cells. The detailed explanation of this scheme as well as a model of the pH-dependent steps in initiation of bacterial DNA replication can be found in (Tiphlova and Karu, 1991b).

It is evident that the irradiation of *E. coli* generates a transient acceleration of cell division reflecting greater metabolic activity only in the cells whose rate of growth is slow. The irradiation also increases the ability of *E. coli* cells to promote the growth of unexposed bacteriophage T4 (Tiphlova and Karu, 1989).

3.2. The Activation of Yeast Metabolism

The effect of the irradiation on the yeast cultures can also be evaluated by the growth curves. Samples irradiated in buffer (Section 2.2) as well as control ones (kept in the dark for the same time) were transferred to 50-ml rocher mounted flasks with nutritive medium and grewed by intense aeration for time intervals from 2 to 18 h as described in Section 2.1.2. The individual portion of cells was irradiated and incubated for every data point shown in Figures 4-7. The number of cells and buds as well as the amount of synthesized protein were measured as described in (Fedoseyeva *et al.*, 1984, 1988a, b). As seen in Figure 4A, the cells of the exposed culture of *S. magnusii* reach the stationary phase of growth about 1.5 h earlier than the unexposed one. Similar regularity (no changes in duration of the lag-period and increased growth in the log-period) is valid in case of other yeasts also (Karu, 1989b; Fedoseyeva *et al.*, 1988a).

Figure 4B presents the increase in number of cells and buds in non-irradiated and irradiated with He-Ne laser *T. sphaerica*. The growth curves show that the duration of lag period is practically the same in irradiated and control culture. In the logarithmic phase of the growth the average generation time decreases by 1.5-1.8 times in the exposed yeast (Fedoseyeva *et al.*, 1984).

The increased accumulation of biomass of the exposed culture was followed by a strictly proportional increase in the number of cells and buds in the logarithmic phase of growth (Fedoseyeva *et al.*, 1984). It follows from the point that the size of the cells and the amount

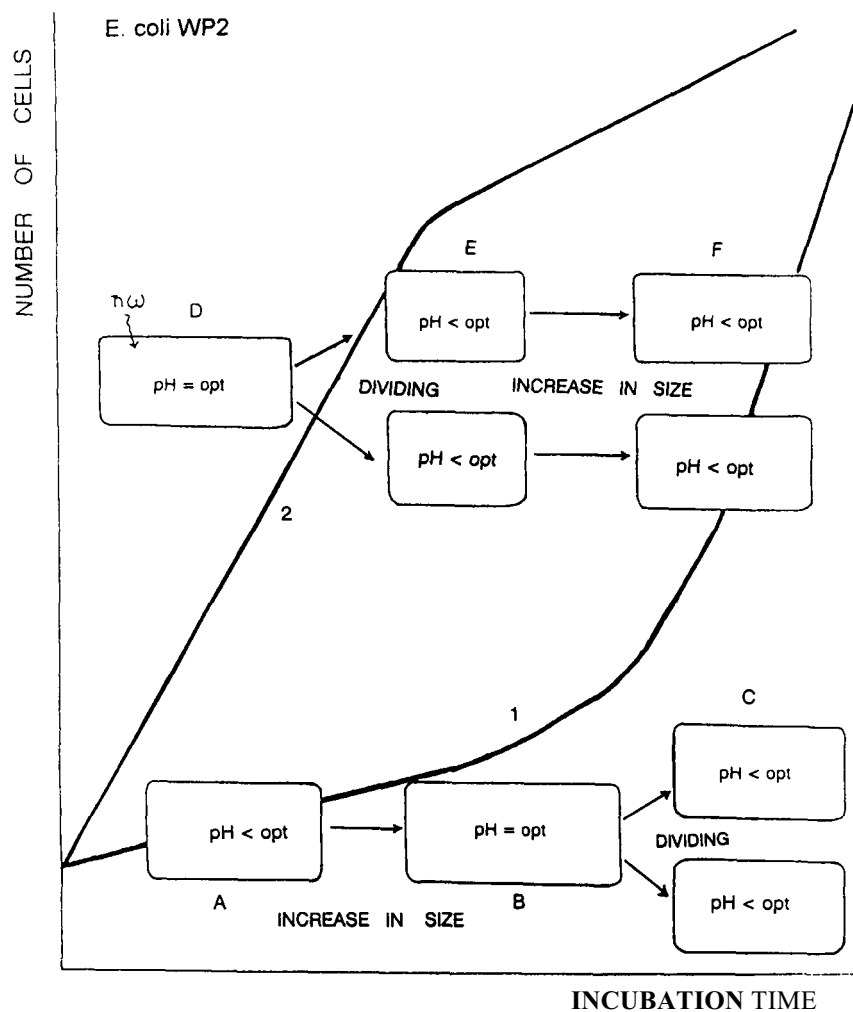


FIGURE 3 Schemes explaining the growth curves of (1) control and (2) irradiated *E. coli* cultures. In the case of nonirradiated control culture (curve 1), an increase of the cell size (A) during the lag period leads to a proper pH gradient and, thus to the initiation of division (B). After the division, the two daughter cells have lowered pH and cannot initiate replication immediately (C). The activation of the respiratory chain by irradiation can shift the equilibrium in pH formation and utilization into the direction of increased pH production (Tiphlova and Karu, 1991b) without increasing the cell size (D). That gives the irradiated cells the possibility of dividing immediately, resulting in two cells with reduced size (E). During the following period, when these cells do not divide, they achieve a size similar to the control cells (F). A detailed description of the pH-dependent division mechanism can be found in Tiphlova and Karu, 1991b.

ACTIVATION OF METABOLISM WITH LIGHT

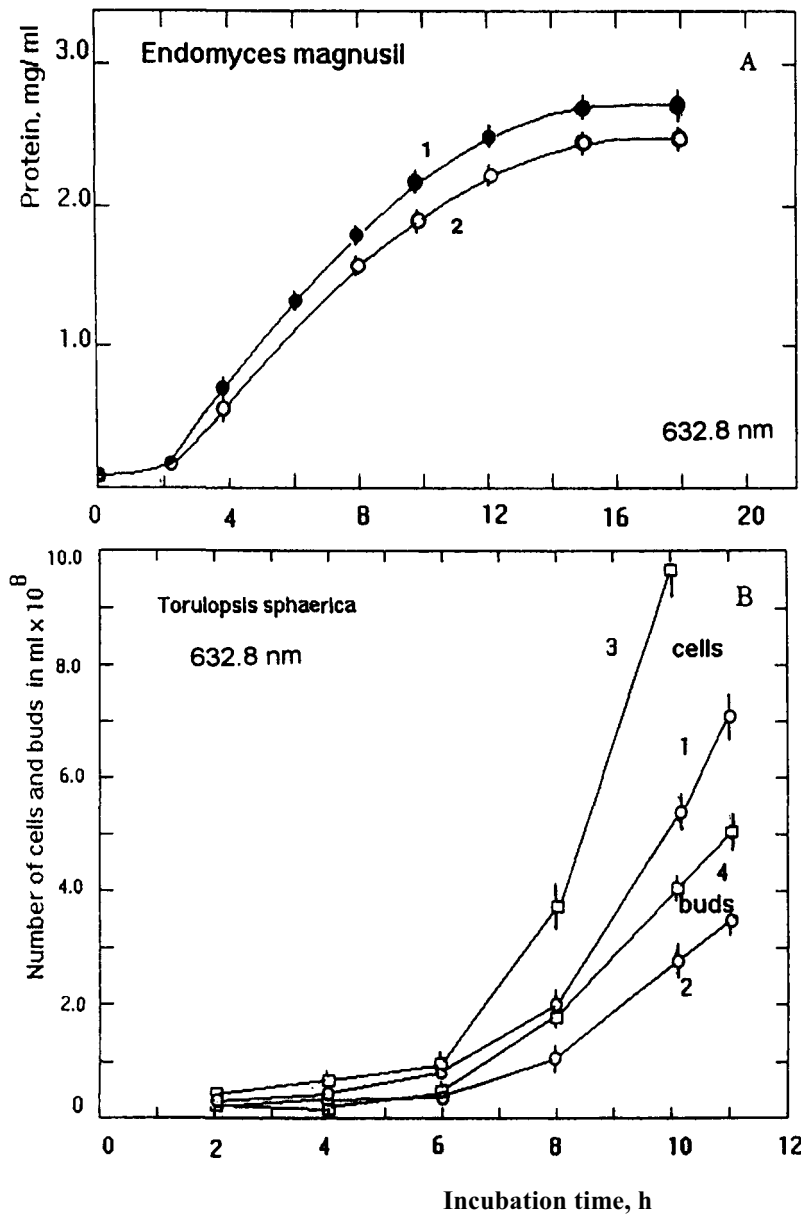


FIGURE 4 (A) Growth curves of *Endomyces magnusii* (measured as a change in amount of synthesized protein as described in Fedoseyeva *et al.*, 1984): 1-culture irradiated with He-Ne laser in dose $6.3 \times 10^3 \text{ J/m}^2$, 2-control culture. (B) Growth curves of *Torulopsis sphaerica* (measured as changes in the number of cells and buds as described in Fedoseyeva *et al.*, 1984, 1, 2- control cultures, 3, 4- cultures irradiated with a He-Ne laser at the dose of $4.2 \times 10^3 \text{ J/m}^2$).

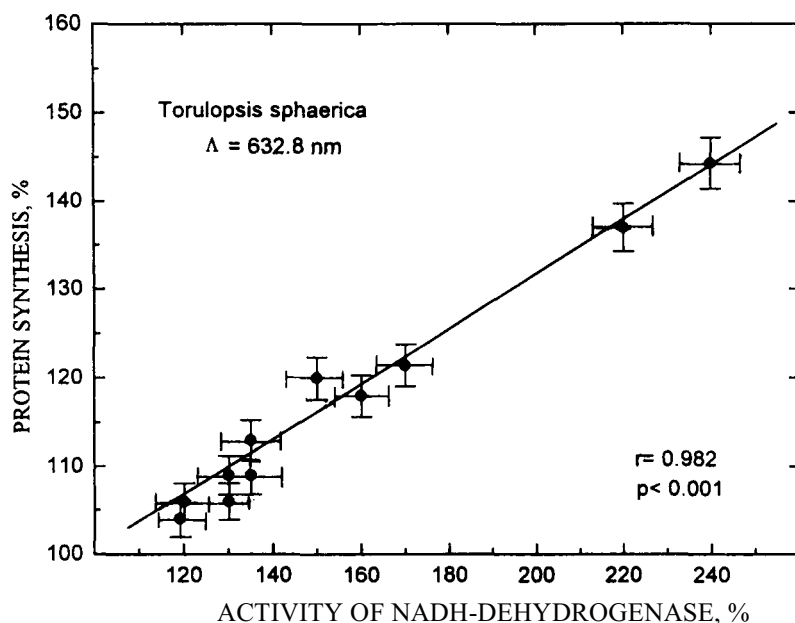


FIGURE 5 Correlation of the activity of NADH-dehydrogenase and the amount of synthesized protein in *Torulopsis sphaerica* irradiated with a He-Ne laser in various doses from 2×10^2 to 1.9×10^3 J/m² as described in Fedoseyeva *et al.*, 1988b.

of protein in the single cell apparently do not differ for exposed and unexposed cultures. Thus, the irradiation with He-Ne laser leads to intensification of protein synthesis and speeds up the preparation of cells for division and budding.

Metabolic processes in irradiated yeast are intensified as summarized in Table IV. The decrease of activity of acid phosphatase, the marker enzyme of lysosomes responsible for hydrolytic activity in a cell, suggests that synthetic processes prevail over degradative processes in the irradiated cells. This shift to the synthetic processes is characteristic of actively multiplying cells (Fedoseyeva *et al.*, 1988b).

The increase in activity of respiratory chain enzymes NADH-dehydrogenase and cyt. c oxidase (Table IV) is consistent with the enhanced O₂ consumption by the irradiated cells (Fedoseyeva *et al.*, 1988b). There exists a correlation between the activation of protein synthesis and activity of NADH-dehydrogenase in the irradiated cells (Fig. 5).

Even though the reaction of various yeasts to the irradiation is qualitatively similar, there may be essential quantitative differences. For instance, the amount of protein in the irradiated culture of *T. sphaerica* at the end of the exponential growth phase is almost double that of the unirradiated culture, but for *E. magnusii*, only 130% (Fig. 6) (Fedoseyeva *et al.*, 1984). Dose-dependences of protein synthesis stimulation for various yeasts also illustrate this conclusion: in some cases the maximal effect is 240-250%, but in other cases, only 110-120% (Fig. 7). The problem of optimal light parameters for irradiation will be considered in Section 4.3.

ACTIVATION OF METABOLISM WITH LIGHT

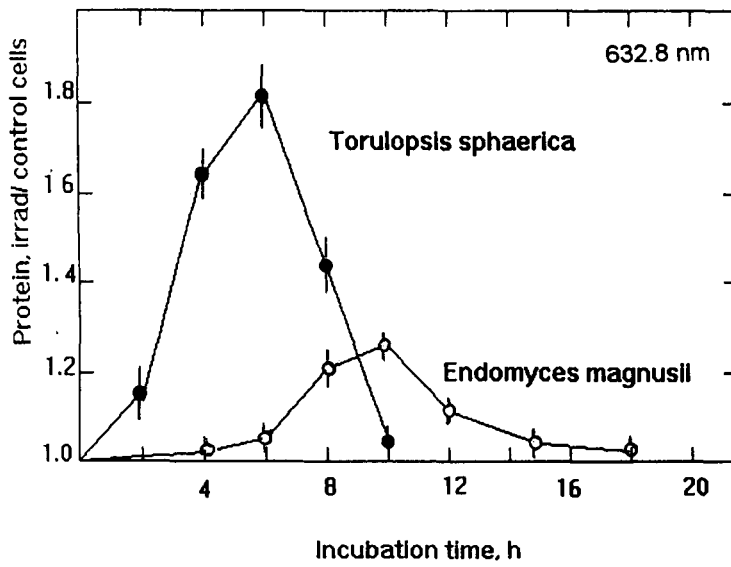


FIGURE 6 The ratio of the growth of control and exposed to He-Ne laser radiation (doses of 4.2×10^3 J/m^2 and 6.3×10^3 J/m^2 , respectively in case of *T. sphaerica* and *E. magnusii*) evaluated from the quantity of synthesized protein as described in Fedoseyeva *et al.*, 1984.

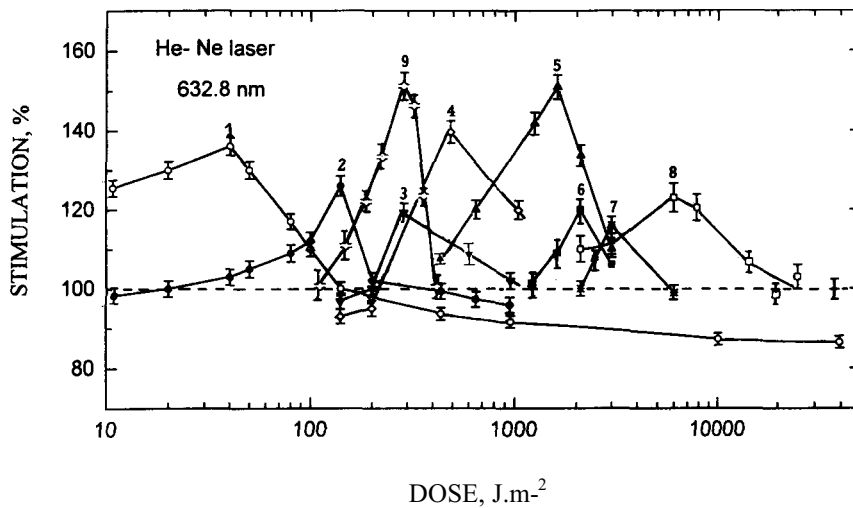


FIGURE 7 Amount of synthesized protein as a percentage of control level measured 18 h after the irradiation with a He-Ne laser in various doses of following yeasts: (1) *Saccharomyces ludwigii* (grown with glucose as a carbon source), (3) *Candida boidinii* (glucose), (4) *Torulopsis sphaerica* (glucose), (5) *Candida boidinii* (meth-anol), (6) *Candida maltosa* (glucose), (7) *Saccharomyces cerevisiae* 14 (glucose) (Fedoseyeva *et al.*, 1988a), (8) *Endomyces magnusii* Fedoseyeva *et al.*, 1984). Curve (2) denotes the dose-dependence for *Saccharomyces ludwigii* grown in anaerobic conditions (Kani *et al.*, 1993a) and curve (9) depicts the dose dependence for endospore germination and outgrowth of *Anaerobacter polyendosporus* (Kutomkina *et al.*, 1991).

TABLE IV

Changes in the activity of enzymes of oxidative and phosphorous metabolism in irradiated with He-Ne laser ($4.6 \times 10^2 \text{ J/m}^2$) yeast *T. sphaerica* (18 h after the irradiation)

Enzymes	Location in the cell	Activity (% of control value)
NADH-dehydrogenase	Mitochondrion	241.2 ± 10.8
Cyt. c oxidase	Mitochondrion	121.4 ± 9.6
Acid phosphatase	Lysosome	48.1 ± 6.3
Catalase	Peroxisome	75 ± 2
Superoxide dismutase	Cytoplasm	103 ± 3

Experimental details are described in Fedoseyeva *et al.*, 1988b.

Thus, the irradiation of yeasts intensifies protein synthesis and speeds up the preparation of cells for division and budding. In contrast to the culture of *E. coli*, yeasts did not show any reduction of the lag period. The growth stimulation in yeasts manifested itself as a shortening of the average generation time (and thus, enhanced accumulation of biomass) in the logarithmic period of culture growth.

3.3. Irradiation-Induced Germination of Endospores

To evaluate the effect of irradiation, the number of germinated and outgrown endospores was compared between control and He-Ne laser irradiated samples. 2×10^6 endospores of *Anaerobacter polyendosporus* were plated on every Petri dish (Kutomkina *et al.*, 1991).

The number of germinated and outgrown endospores (counted as the number of formed colonies) was 300 ± 25 (0.015%) in control experiments. The effect of irradiation manifested itself in the increase of the number of germinated and outgrown endospores. As seen in Figure 7 (curve 9), the number of outgrown endospores depends on the irradiation dose, starting to increase at higher doses than 150 J/m^2 and being maximal (1.5 times higher than in the intact control) at 300 J/m^2 . Further increase in the dose caused a decrease of the number of outgrown endospores, being on the control level at the dose near 450 J/m^2 .

Thus, it was found that the irradiation with He-Ne laser at particular doses increased the number of germinated and outgrown endospores. It is interesting to recall that this kind of bell-shaped dose dependence is characteristic for various types of low-power laser effects (Fig. 7, and also Karu, 1989b).

The molecular mechanism of germination is not fully understood. The mechanism of activation of germination by light is not clear either. At the moment, we can only conclude that the irradiation with light at 632.8 nm and at dose range from 150 to 450 J/m^2 increased the number of germinated and outgrown endospores of *Anaerobacter polyendosporus*.

4. POSSIBLE MISTAKES, DIFFICULTIES WHICH SHOULD BE TAKEN INTO CONSIDERATION

4.1. The Culture and Its Growth Conditions

As it was illustrated in Figure 2, in case of *E. coli* WP2 trp^- the effect of growth stimulation manifested itself in specific growth conditions (Tiphlova and Karu, 1991b). It was found later that only those strains of *E. coli* whose normal rate of growth was particularly slow

due to the presence of factors inhibiting cell reproduction or with alterations in metabolic pathways, can be stimulated (Bertoloni *et al.*, 1993). The same authors were not able to stimulate a fast-growing wild strain of *coli* (G. Bertoloni and G. Jori, personal communication). Recall also that the photostimulation was markedly more effective on bacterial cells during the exponential phase of growth as compared with cells in the stationary phase (O. Tiphlova, T. Karu, unpublished; Bertoloni *et al.*, 1993).

In case of yeasts, the activation percentage of protein synthesis was found to be culture-dependent (Fedoseyeva *et al.*, 1988a). The more photosensitive cultures (a lower dose range needed for activation) were activated to a greater degree (Fig. 7). The cultures with more intense respiration were activated to a higher degree, the amount of synthesized protein was larger after the irradiation with the optimal dose (Fedoseyeva *et al.*, 1988a). In our experiments with yeasts, we found that optimal doses were in agreement with the degree of lability of their metabolism, i.e. with the possibility of restoring normal metabolism (Fedoseyeva *et al.*, 1988a). For example, *Saccharomyces cerevisiae* 14 is characterized by a rather conservative type of metabolism, required a very large light dose for activation. At the same time, the stimulation with the optimal irradiation conditions was the lowest among yeasts studied, $116 \pm 3\%$ (Fedoseyeva *et al.*, 1988a). Clearly, *Saccharomyces cerevisiae* is not the best choice for irradiation experiments as was also determined by (Quickenden and Daniels, 1993). On the contrary, *Saccharomyces ludwigii* and *T. sphaerica* with very labile possibilities for accommodation, appeared to be activated by low doses of red light (Fig. 7).

To demonstrate how reconstruction of cellular metabolism influences the photosensitivity, we performed experiments with *C. boidinii* grown on the Reder medium with glucose or methanol as carbon sources (Fedoseyeva *et al.*, 1988a). For the yeast grown on methanol (those cells have a glyoxylatic type of respiration), the optimum dose ($\lambda = 632.8$ nm) was higher than the optimal dose for glucose-grown yeast (1.5×10^3 and 3×10^2 J/m², respectively; curves 5 and 3 in Fig. 7), and the amount of the protein synthesized under action of the optimum dose was higher, also ($151 \pm 5\%$ and $122 \pm 1\%$, respectively).

4.2. Irradiation Procedure

The main important features of the irradiation procedure were described already in Section 2.2. Recall briefly the possible sources of mistakes: extraneous illumination (sunshine or room lights) during the experiment, high density of suspension of microorganisms, too thick layer of suspension, suspension with meniscus and stirring of budding yeasts. All of these mistakes were made by Quickenden and Daniels (1993), and Daniels and Quickenden (1994). These programs can serve as examples to students how not to irradiate cell suspensions. Last but not least, the parameters of light (wavelength, dose, intensity, pulse repetition rate and/or pulse duration in case of pulsed light) should be the "right ones." In Section 4.3, it will be shown how the stimulative effects of irradiation depend on the light parameters.

4.3. Irradiation Parameters

For each culture there exists a proper *dose* range, which can be different for different wavelengths (see Karu, 1989b), as was already illustrated for yeasts when irradiating at 632.8 nm (Fig. 7). Exposure to lower or higher light doses does not produce the stimulating effects.

According to the reciprocity law, photobiological response is independent of the *intensity* of light and *irradiation time* when the dose is kept constant. It was found that when

irradiating *E. coli* WP2 at 454 nm (Tiphlova and Karu, 1988,1991b), or HeLa culture at 633 nm (Karu, 1989b), the reciprocity law was invalid. The effects were found to depend on the light intensity and irradiation time, with the dose being constant. Later, two different maxima in the dose-dependence curves of *E. coli* WP2 growth stimulation (Karu *et al.*, 1994) were found as shown in Figures 8 and 9. In the case of the first maximum (A, Fig. 9), the reciprocity law is obeyed, and in the case of the second maximum (B, Fig. 8), it is invalid. In other words, the maximum A depends on the irradiation time, the maximum B being independent of it (Fig. 9). For the first stimulation maximum (A) to occur the cells must be exposed to a dose of $50 \cdot 10^2 \text{ J/m}^2$. For the second maximum (B), it is necessary to irradiate for at least 100 s, no matter what the irradiation intensity is (Karu *et al.*, 1994).

In case of pulsed light, the irradiation effects also depend on *pulse repetition rate* and/or *pulse duration*. These effects are studied more carefully for mammalian cells (Karu *et al.*, 1993c, d). For *E. coli* WP2 growth stimulation, the data exists for femtosecond pulses at 620 nm (Karu *et al.*, 1991), nanosecond pulses at 890 nm (Zharov *et al.*, 1987) and milli- and

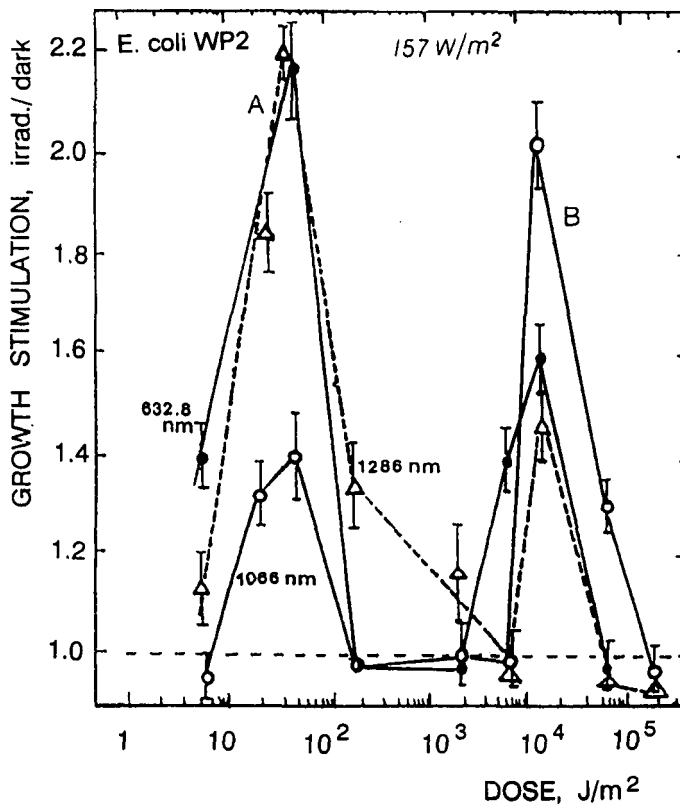


FIGURE 8 Dose dependence for *E. coli* WP2 growth stimulation. The bacterial suspension was irradiated at an intensity of 157 W/m^2 and wavelengths of 632.8, 1066 and 1286 nm as described in Kara *et al.*, 1994.

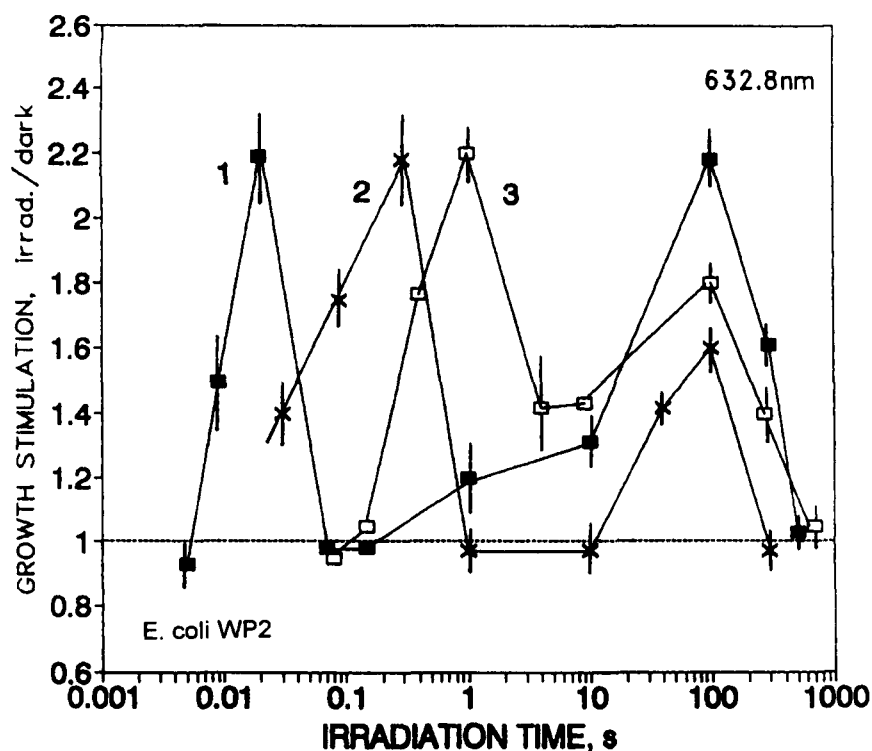


FIGURE 9 *E. coli* growth stimulation as a function of the irradiation time. The bacterial suspension was irradiated at 632.8 nm and intensity of (1) 3×10^3 W/m², (2) 157 W/m², (3) 60 W/m² as described in Kara *et al.*, 1994.

microsecond pulses at 950 nm (Karu *et al.*, 1990). Figure 10 presents an example off. *coli* WP2 growth stimulation when irradiating at 950 nm.

Figure 11 illustrates the dependence of irradiation effect (growth stimulation off. *coli* WP2 *trp*⁻ and protein synthesis stimulation of *Saccharomyces ludwigii*) on the wavelength of continuous wave light (action spectra). As seen in these two action spectra, there are maxima practically in every band of visible light, as well as their exist, but there are also wavelengths which are not effective.

Summing up, one should emphasize that in light-induced growth stimulation experiments, it is as necessary to find the right parameters of light as it is to choose the "right culture" and the "right cultivation conditions."

4.4. When to Measure the Irradiation Effect

Biochemical and morphological changes in the irradiated cells depend on the time elapsed after the irradiation (Section 3). This means that the measurements should be made at the "right" time. For example, a significant increase in DNA synthesis off. *coli* WP2 *trp*⁻ was detectable

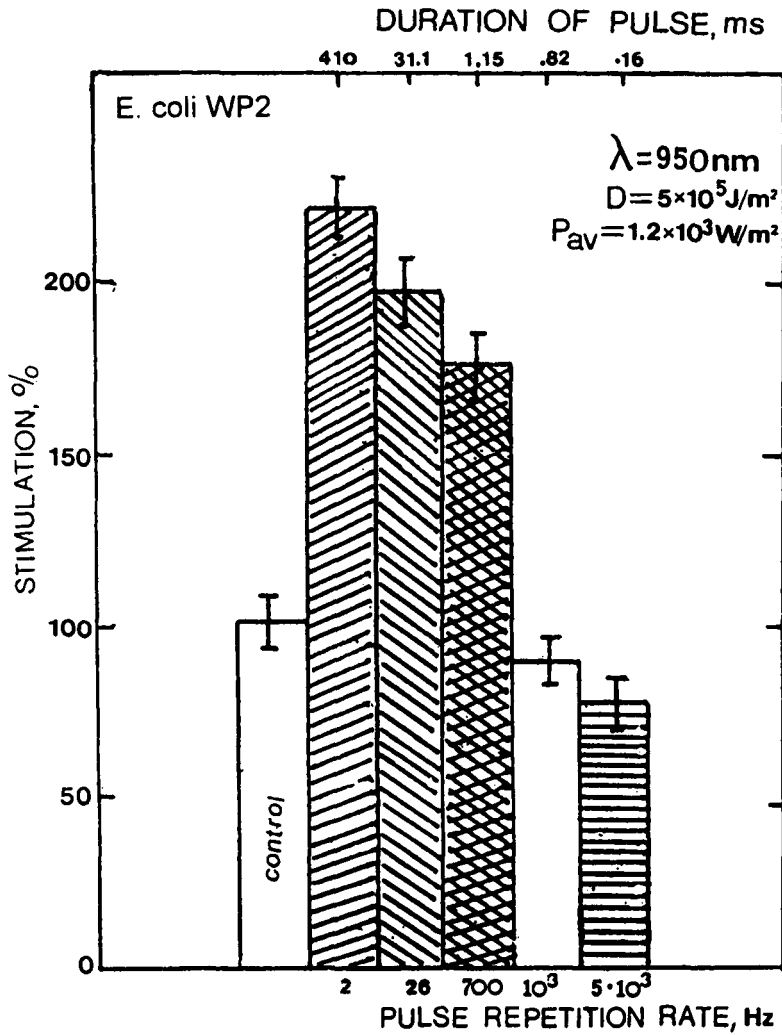


FIGURE 10 Stimulation of *E. coli* WP2 growth after irradiation with a semiconductor laser ($\lambda = 950$ nm, $D = 5 \times 10^5$ J/m², irradiation time 270 s, Karu *et al.*, 1990) as a function of pulse repetition rate and pulse duration.

only during first 10 min after the inoculation (Tiphlova and Karu, 1991b; Karu *et al.*, 1983), and the growth stimulation was maximal 60 min after the irradiation (Tiphlova and Karu, 1988, 1991b; Fig. 2). One should note that we were not able to detect the effect of growth stimulation by spectrophotometric measurements of the density of suspension (Tiphlova and Karu, unpublished). At the same time, the determination of cell number by surface plating technique did show the effect.

When irradiating the yeasts, the irradiation effect was revealed in exponential phase of

ACTIVATION OF METABOLISM WITH LIGHT

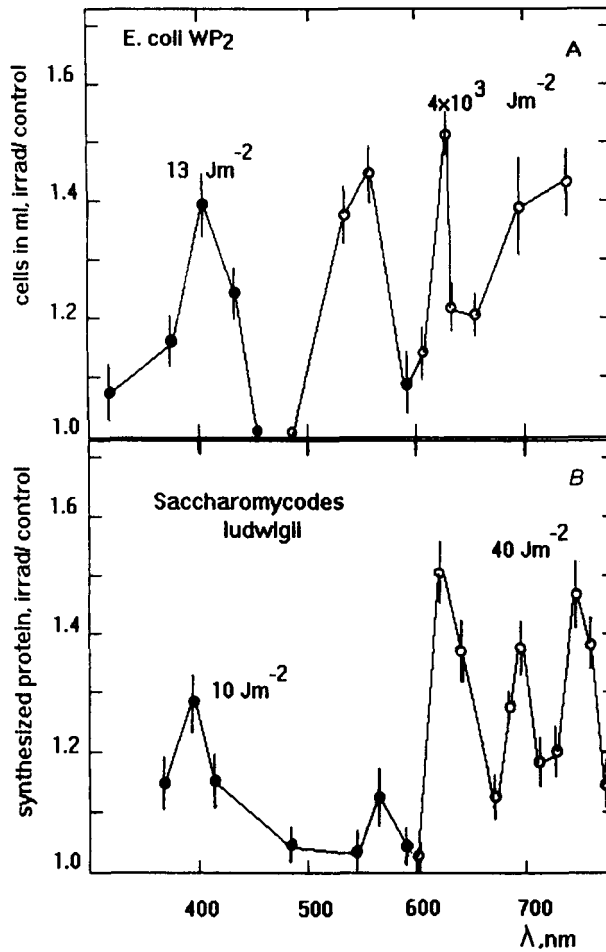


FIGURE 11 Action spectra of monochromatic light upon (A) *E. coli* WP2 growth, and (B) protein synthesis in *Saccharomyces ludwigii*. The experimental conditions are described in Tiphlova and Karu, 1988, and Fedoseyeva *et al.*, 1988a, respectively.

culture growth (Pedoseyeva *et al.*, 1984, 1988a; Fig. 4). At the same time, the growth stimulation effect off. *coli* WP2 was detectable only in lag-phase of growth (Tiphlova and Karu, 1988, 1991b; Karu *et al.*, 1983).

5. PROBLEMS WHICH ARE NOT FULLY UNDERSTOOD

First, the difference between the photosensitivity of *E. coli* K-12 genetically different strains (Voskanyan *et al.*, 1985, 1986; Bertoloni *et al.*, 1993) certainly deserves more

attention in the future. Recall that the sensitivity of bacterial cells to irradiation was proposed to be a pH-dependent genetic process (Tiphlova and Karu, 1991b, also see Fig. 3).

Second, the dependence of the photosensitivity of yeasts on the type of their metabolism (Fedoseyeva *et al.*, 1988a. Section 4.1) is a problem not investigated and explained so far.

Third, in experiments with microorganisms during many years, we found a seasonal variation in maximal possible photostimulation (Karu, 1989b). Figure 12 illustrates this finding. There was practically no growth photostimulation in summer. In winter, the effect was maximum, and in spring and autumn the effect was intermediate.

In case of *E. coli*, this phenomenon was explained in the following way (Karu, 1989b; Tiphlova and Karu, 1991b). In autumn and winter, the culture featured relatively slow growth and then irradiation had a significant effect (e.g., Fig. 2). In spring and summer, when the culture growth accelerated and the growth rate of the control culture was almost comparable to that of the culture exposed to the optimum dose of light in the autumn-winter period, irradiation has but little effect.

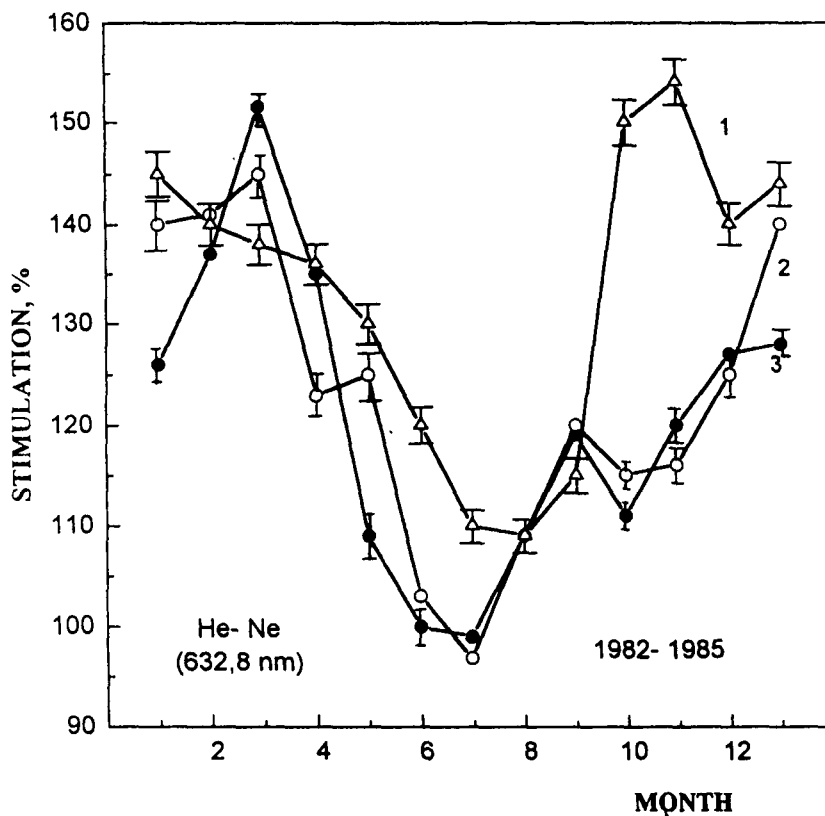


FIGURE 12 Variation of maximal possible growth stimulation of (1) *E. coli* WP2, (2) *T. sphaerica* and (3) *Saccharomyces ludwigii* during year (summary of measurements performed in 1982-1985). The irradiation was performed always at the same time (near 11 a.m. for *E. coli* and near 6 p.m. for yeasts) with a He-Ne laser in doses (1) $4 \times 10^3 \text{ J/m}^2$, (2) $4 \times 10^2 \text{ J/m}^2$ or (3) 40 J/m^2 .

It is quite possible that the seasonal variation of maximal possible photostimulation is connected with existing cellular periodicities and/or solar activity. Some aspects of this problem were discussed in (Karu, 1989b), but the problem clearly needs further investigation.

6. CONCLUSIONS

The protosensitivity of nonphotosynthesizing microorganisms to monochromatic bands of visible light was found to be dependent both on physiological (metabolic) state of cells as well as on light parameters.

In case of *E. coli* WP2 trp⁻ the irradiation generated a transient acceleration of cell division which was expressed in reduction of the latent period of the growth. A model explaining pH-dependent steps in initiation of bacterial DNA replication was proposed (Tiphlova and Karu, 1991b) and a change in intracellular pH (0.32) after irradiation has been actually measured recently (Quickenden *et al.*, 1995). The irradiation also increased the ability of *E. coli* WP2 cells to promote the growth of bacteriophage T4 (Tiphlova and Karu, 1989).

The irradiation of yeasts led to intensification of protein synthesis and speeded up the preparation of cells for division and budding as expressed in shortening of the average generation time in the logarithmic phase of the culture growth. A rearrangement in oxidative and phosphorous metabolism of irradiated cells occurred (Fedoseyeva *et al.*, 1988b; Karu *et al.*, 1993a, b). Some yeast cultures were more photosensitive than others.

The irradiation with a He-Ne laser within a certain dose range increased the number of germinated and outgrown endospores of *Anaerobacter polyendosporus*.

The survival of a microorganism depends upon its ability to adapt and respond to environmental factors. Inasmuch as the visible light is one of the environmental factors, it is not surprising that light-growth responses of microorganisms manifest themselves only in specific conditions and are dependent on light parameters as well as upon the metabolic state of the irradiated cells.

Light-growth responses involve the respiratory chain: the location of primary photo-acceptors (flavins and/or cytochromes) is believed to be here (Karu, 1987, 1988, 1989b, 1990, 1995; Tiphlova and Karu, 1991b). Namely this common biochemical base of the photosensitivity of nonphotosynthesizing cells determines the versatility of the phenomenon. One should recall that photosensitive systems are located not in highly differentiated organs or organelles but are in the light-induced chemical reactions with molecules involved in a metabolic pathway which are rather ubiquitous in nature (Erianger, 1976).

Dedication

I would like to dedicate this paper to the memory of the corresponding member of the Academy of Sciences Professor Maxim Nikolaevich Meissel (1901-1986) who attracted my interest to the life of microorganisms and actively participated in the first experiments with yeasts.

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