

Impact of coherent light on interaction of fungi and bacteria cells cultivated *in vitro*

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Abstract. This article considers impact of coherent red quasi-monochromatic light on interaction of colonies of the *Pseudomonas syringae* bacteria and the *Fusarium macroceras* fungus in an *in vitro* culture. A helium-neon laser and a heat source with a system of light filters and aperture diaphragms were used for irradiation. Two light fluxes were obtained with energy parameters close in magnitude, but significantly different in spatio-temporal coherence. Light with a high statistical ordering stimulated growth of both colonies. Irradiation from the same spectral range and intensity, but with low spatial coherence, increased the functional activity of only small bacteria cells. As a result, there was a suppression of larger fungal cells development that were interacting with them. Therefore, it was the statistical (coherent) properties of light that affected the change in the equilibrium of microorganisms in an artificial biocenosis. This approach can be used in practice for increasing the activity of bacteria antagonists of pathogenic fungi and the non-chemical disease protection of plants.

1 Introduction

Over a long period of coexistence between bacteria and fungi, complex and diverse relationships have been formed: symbiotic, mutualistic, competitive, antagonistic, parasitic and others. These types of contacts allow representatives of these kingdoms to maintain a dynamic balance, which can change under the impact of external and internal factors [1]. In agrotechnology and biotechnology it is often necessary to shift the balance in favor of any of the components of the biocenosis. For example, suppression of fungal pathogens of plant diseases happens through bacteria with antifungal activity [2]. To enhance the effect, we can use factors that selectively affect the cells of certain types of microorganisms. One such factor is quasi-monochromatic irradiation.

Previous studies have shown that interaction of fungi and bacteria can be affected by light of a certain spectral composition and coherence [3]. The experiments were carried out *in vitro* on fungal colonies infected with bacteria. Mushrooms release metabolic products (amino acids, peptides, sugars, etc.) out in the environment that are attractive to bacteria [4, 5]. As a result, they concentrate near and on the surface of the hyphae and do not adversely affect

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them to a certain level. Short-term exposure to low coherent irradiation in the red spectrum range led to inhibition of the fungal colonies. Highly coherent light with the same energy parameters, on the contrary, stimulated their development [3].

The marked change in the functional activity of cells is associated with photoregulatory processes. Taking into account the wavelength of the exciting irradiation (634 nm), we can speak of a reaction mediated by phytochrome B (phyB). It is present in bacteria, fungi and plants. Under the action of red light, a photoconversion of this chromoprotein in the far-red form (Pfr) occurs and the chemical signaling cascade is triggered. As a result, many endogenous processes are enhanced, up to gene expression [6, 7]. However, these processes turned out to be dependent on the statistical ordering (coherence) of the exciting light. The greatest stimulating effect occurred when the cells were completely placed in the volume of coherence of the field of a quasi-monochromatic wave [8]. This can explain the change in equilibrium in the model biocenosis with small bacterial cells and larger fungal cells when exposed to light with different coherence.

There is a dynamic equilibrium depending on the spectral composition of the acting irradiation between the active Pfr and the passive Pr forms of phyB [9]. In the considered experiments [3], the statistical ordering of the field of the light wave was formed by means of certain values of spatial and temporal coherence. In the latter case, the spectral line width was changed, which could affect phyB photoconversion. The following questions arise: did the spectral or the correlation properties of irradiation affect cell interaction? How will light coherence act on fungal and bacterial cell colonies when they are cultured together? This work is dedicated to answer these questions.

2 Materials and methods

The biological material of our study was the colonies of the *Fusarium macroceras* fungus and the *Pseudomonas syringae* bacterium. They were cultivated in Petri dishes both separately and together for 7 days and at a temperature of 22 °C. During joint cultivation, the colonies were seeded at a distance of 2.5 cm from each other. The nutrient medium was a potato-glucose agar containing 1% glucose and 1% agar. The sizes of bacterial cells are 2-5 μm, the sizes of fungal cells are 8-14 μm. Irradiation was performed 24 hours after plating on the culture medium. The results of quasi-monochromatic light were evaluated by the area or volume of colonies on the 7th day of cultivation. The experiments were repeated six times. The histograms show the average values and the errors.

A helium-neon laser (λ_{\max} 632.8 nm) was used as a source of highly coherent light, which provided the following statistical field parameters: the coherence length L_{coh} and the correlation radius r_{cor} of more than 200 μm. Light with less spatiotemporal coherence was obtained from a heat source with a system of light filters and aperture diaphragms. In this case, $\lambda_{\max} = 634$ nm, $L_{\text{coh}} = 140$ μm, $r_{\text{cor}} = 6$ μm. The energy characteristics of the obtained quasi-monochromatic light beams differed within the measurement error (less than 5%). The power density was 1 W/m², the wavelength at the maximum of the spectral line was 633–634 nm, and the irradiation duration was 240 seconds. Control variants of the experiment were not exposed to quasi-monochromatic light. The irradiation power density was determined by a “VEGA” laser irradiation meter (Ophir, Israel), and the transmission spectra of interference filters were measured on an “Analytik Jena Specord 250 Plus” spectrophotometer (Germany) with an accuracy of 0.5 nm. The analytical system for calculating the characteristic values of temporal and spatial coherence is presented in [10, 11].

3 Results and discussion

When using a monoculture of the fungus *F. macroceras* and *P. syringae* bacteria, short-term (240 seconds) laser irradiation led to an increase in the functional activity of microbial colonies. After 7 days, their areas in relation to control variants increased by 1.6 and 3.3 times, respectively (Fig. 1, 2). The experiment showed a rather high sensitivity of the selected microorganisms to red light, which made it possible to use them to elucidate the impact of the statistical properties of irradiation on interaction of cells.

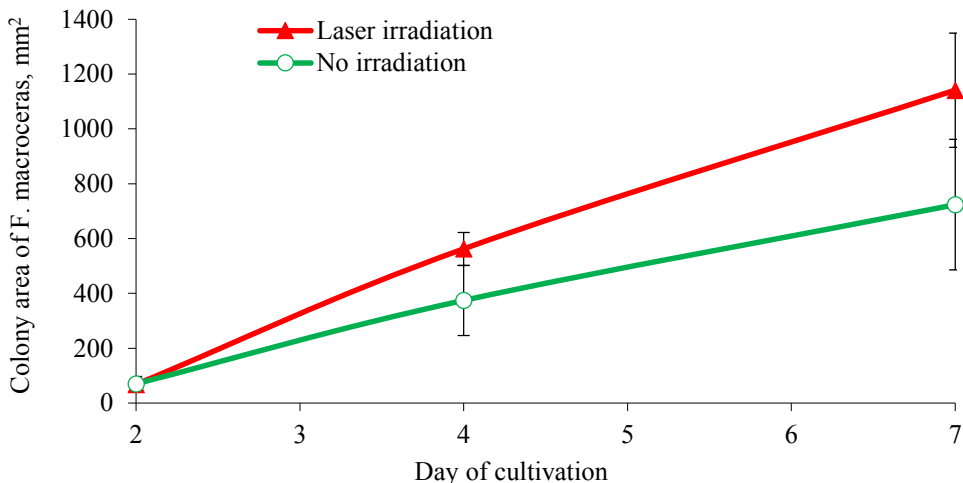


Fig. 1. Impact of laser (632.8 nm) irradiation on colonies growth of the *F. macroceras* fungus on an artificial growth medium.

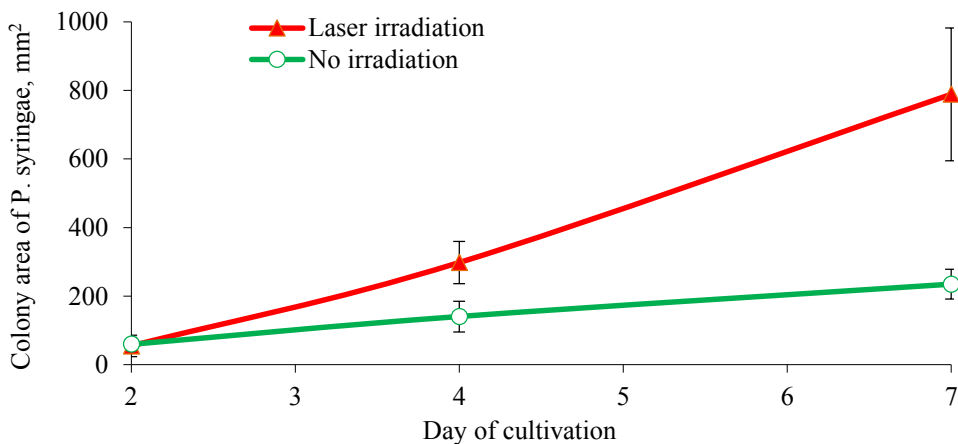


Fig. 2. Impact of laser (632.8 nm) irradiation on colonies growth of the *P. syringae* bacteria on artificial growth medium.

In another embodiment, *F. macroceras* or *P. syringae* were grown in both mono and double culture. They were irradiated with light with high or low statistical ordering 24 hours after plating. Red quasi-monochromatic light increased the functional activity of separately cultivated bacteria. This is especially noticeable with high irradiation coherence (Fig. 3, left). The presence of fungal colonies on the nutrient medium inhibited the growth of *P. syringae* and prevented development of a stimulatory effect (Fig. 3, right).

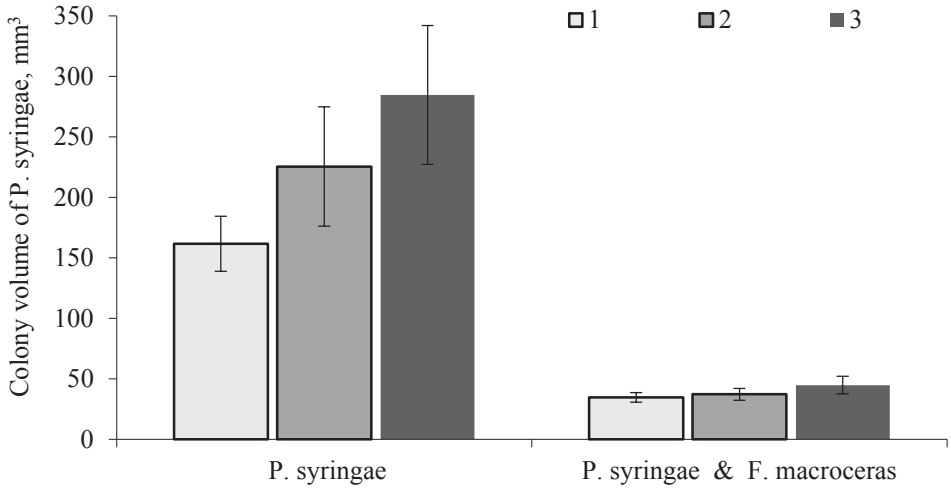


Fig. 3. Impact of coherent light on colonies growth of the *P. syringae* bacteria in the absence of the *F. macroceras* fungus colonies (left) and during their joint cultivation (right). 1 – no exposure; 2 – irradiation with low coherent light $L_{coh} = 140 \mu\text{m}$, $r_{cor} = 6 \mu\text{m}$; 3 – irradiation with highly coherent light (L_{coh} , $r_{cor} > 200 \mu\text{m}$).

A more complex situation was observed during cultivation of *F. macroceras*. Low coherent light suppressed the development of fungal colonies. 6 days after irradiation, their volume was one and a half to two times less than in the control variants (Fig. 4). The need to take into account not the area, but the volume of the colonies, is associated with a significantly different growth of aerial mycelium in several varieties of this experiment.

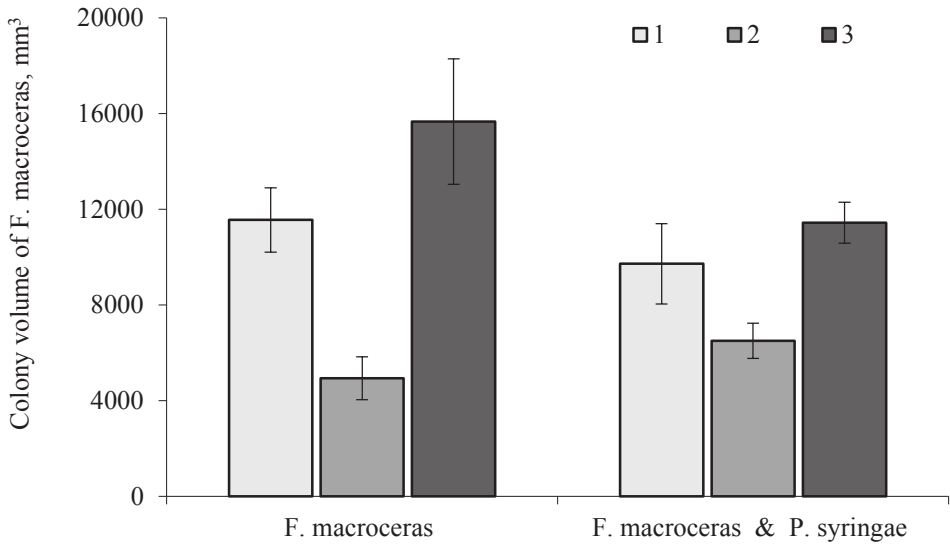


Fig. 4. Impact of coherent light on colonies growth of the *F. macroceras* fungus in the absence of the *P. syringae* bacteria colonies (left) and during their joint cultivation (right). 1 – no exposure; 2 – irradiation with low coherent light $L_{coh} = 140 \mu\text{m}$, $r_{cor} = 6 \mu\text{m}$; 3 – irradiation with highly coherent light (L_{coh} , $r_{cor} > 200 \mu\text{m}$).

Highly coherent light stimulated the development of *F. macroceras*, both as separate (Fig. 4, left), and in combination with bacterial colonies. In the latter case, the difference with the benchmark was not significant (Fig. 4, right).

In order to explain the obtained results, it is necessary to compare the cell sizes of microorganisms with the characteristic coherence parameters of the used light beams. It was previously established that the stimulation effect is most pronounced when the cell is placed completely in the volume of field coherence, that is, $D < L_{coh}, r_{cor}$, where D is the cell size [8]. In the case of *P. syringae* (small cells), this condition was satisfied for both sources of quasi-monochromatic irradiation, and the functional activity of bacteria increased. This was manifested depending on the cenotic ambience. During joint cultivation, the fungus metabolites suppressed the bacterial colonies' growth and the photoregulatory effect was smoothed out (Fig. 3, right). On the larger cells of *F. macroceras*, a positive effect occurred only in the case of highly coherent laser irradiation (Fig. 4). Low coherent light, on the contrary, inhibited fungal growth, which may be due to the predominant stimulation of *P. syringae* and an increase in the concentration of metabolites with antifungal action. This also happened with a separate cultivation of *F. macroceras* colonies (Fig. 4, left), which indicates their infection with the bacteria (Fig. 5).



Fig. 5. Localization of the *P. syringae* bacteria around the *F. macroceras* hyphae.

The spectral lines were very narrow (no more than 3 nm) for low and high coherent light fluxes, which is ten times smaller than the phyB direct photoconversion spectrum. In this case, the same reaction of large and small cells could be expected, especially since the coherence length was sufficient enough to fulfill the above inequality. But that did not happen. The reason for that is probably related to spatial coherence, which was significantly different for the used flows. Therefore, not the spectral, but the statistical (coherent) properties of the irradiation caused the change in the intensity of the photoregulatory processes in the experiment.

4 Conclusion

Insufficient attention is being paid to the influence of light coherence on photoregulatory processes. The reason is probably related to the complexity of understanding of the phenomenon at the intersection of physics and biology. However, it provides abundant opportunity to control the functional activity of living organisms. This is most clearly manifested in the interaction of cells of varying sizes. By changing the parameters of coherence, it becomes possible to selectively stimulate smaller cells without affecting the functional activity of the larger ones. On the other hand, the use of highly coherent irradiation, for example, laser irradiation, makes it possible to increase the activity of almost any cell. Naturally, the wavelength of quasi-monochromatic light should correspond to the spectrum of action of any of the photoregulatory reactions.

The described experiment confirms the abovementioned ideas. The coherence of light significantly affected the interaction of the *P. syringae* bacteria and the *F. macroceras* fungus during their joint cultivation in vitro. Light with high statistical ordering stimulated the growth of both colonies. Irradiation from the same spectral range and intensity, but with low spatial coherence, increased the functional activity of only small bacteria cells. As a result, there was a suppression of development of larger fungal cells interacting with them. Thus, it was the statistical (coherent) properties of light that affected the change in the equilibrium of microorganisms in an artificial biocenosis. This approach can be used in practice for increasing the activity of bacteria antagonists of pathogenic fungi and the non-chemical disease protection of plants.

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