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EXPERIMENTAL ARTICLES

Effect of Bacterial Satellites on *Chlamydomonas reinhardtii* Growth in an Algo–Bacterial Community

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Abstract—The growth characteristics of an algo–bacterial community (*Chlamydomonas reinhardtii* and bacterial satellites) were studied, as well as the mechanism and patterns of bacterial effect on algae. Four strains of predominant bacteria were isolated and partially characterized. They were assigned to the following taxa: *Rhodococcus terrea, Micrococcus roseus*, and *Bacillus* spp. A pure culture of the alga under study was obtained by plating serial dilutions on agarized media. Within the algo–bacterial association, the alga had a higher growth rate (0.76 day^{-1}) and yield (60 μg chlorophyll/ml culture) than in pure cultures (0.4 day^{-1} and 10 μg chlorophyll/ml culture, respectively). The viability of the algal cells within the association was retained longer than in pure culture. Among the isolated bacterial satellites, strains B1 and Y1, assigned to the species *Rhodococcus terrae*, had the highest stimulatory effect on algal growth. The culture liquid of bacteria incubated under the conditions not permitting growth stimulated algal growth; the culture liquid of actively growing bacteria had an opposite effect.

Key words: algo–bacterial community, *Chlamydomonas reinhardtii*, growth stimulation, mechanisms of interaction.

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Algo–bacterial communities are a convenient model for investigation of such aspects of ecophysiology as producer–consumer interactions and interspecies communication and cooperation. Most of the work on associations of algae and their bacterial satellites has dealt with their trophic relationships [1–3] and mutual suppression or cooperation [3, 4]. Communication between algae and bacterial satellites via homoserine lactones and other low-molecular regulators has recently become a popular issue [5, 6]. In such communities, algae are the only source of nutrient supply into the system; the relations between algae and the organisms constituting the upper levels of the food chain are therefore mainly trophic. However, bacterial components of a balanced community depend on the state and activity of the algae; therefore, bacterial satellites require some means to affect the algae. Few publications consider the stimulatory effect of bacteria on algae [3]. The mechanisms of this effect are diverse and not always clear. Bacteria increase CO_2 content in the medium and decrease oxygen concentration; these changes are favorable for algal growth and increase their assimilation coefficient [7]. Bacteria can also facilitate mineralization of difficult-to-degrade compounds, enrich the medium with nitrogen salts [8], and synthesize vitamins and auxins [9]; these can be other effective factors.

The goal of the present work was to investigate the nature of interactions in the association of a unicellular alga *Chlamydomonas reinhardtii* and bacterial satellites.

MATERIALS AND METHODS

The algologically pure culture of *Chlamydomonas reinhardtii* Dang. (*Chlorophyta*, *Chlorophyceae, Volvocineae*) was obtained from the Department of Biophysics, Moscow State University. It is a green unicellular alga with two flagella; the cells are of regular spherical or, less often, ellipsoidal shape. This is a typical α-mesosaprobic organism; it occurs in nature in the environments moderately contaminated with organic compounds (pools, ponds, bogs, lakes, and rivers) [10–13]. The hanging drop technique was used for prolonged observations of algal development [10]. A drop of the culture was placed on a clean coverslip; the coverslip was then placed on a well slide and sealed with

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paraffin. During the intervals between observations the preparations were stored in a moist chamber. The experiments were carried out for one month.

The culture was maintained on a modified HS medium containing the following (g/l): KH_2PO_4, 1.0; K_2HPO_4, 1.3; NH_4Cl, 0.54; $MgSO_4 \cdot 7H_2O$, 0.12; sodium citrate, 0.32; $CaCl_2 \cdot 2H_2O$, 0.05; $FeCl_3 \cdot 6H_2O$, 0.003; and Hunter microelement mixture [14]. Microscopy revealed bacterial satellites in some of the *C. reinhardtii* cultures. In order to obtain a bacteriologically pure algal culture, a combination of ampicillin selection (2500 U/ml) and the exhausting streak method [15] was used. The pure *C. reinhardtii* culture was maintained on Pratt medium containing the following (g/l): KNO_3, 0.10; K_2HPO_4, 0.01; $MgSO_4 \cdot 7H_2O$, 0,01; $FeCl_3 \cdot 6H_2O$, 0.001; and agar, 12.0 [12]. For the isolation of bacteria, PYG medium was used containing the following (g/l): glucose, 5.0; peptone, 5.0; and yeast extract, 5.0; the pH was 7.0. M9 medium [16] supplemented with carbon sources and liquid Raymond medium supplemented with diesel fuel were used to determine the growth requirements of bacterial isolates. The sensitivity to the egg lysozyme preparation was determined by the terminal dilution method [17].

The chemical composition of the cell wall of asporogenous bacteria was determined by the standard methods [18]. In order to determine the differentiating sugars and to obtain methylmycolates, the strains were cultivated with shaking (150 rpm) on nutrient broth for 96 h at 27°C. For determination of *meso*-diaminopimelic acid (DAPA), the cultivation was carried out on slanted nutrient agar for 48–72 h at 27°C. The DAPA isomers were determined in whole cell hydrolysates by ascending chromatography in the following solvent system: methanol : distilled water : 6 N HCl : pyridine (80 : 26 : 4 : 10). To develop the chromatograms, they were sprayed with 0.5% ninhydrin in acetone, dried, and heated for 2 min at 105°C.

Mycolic acids were determined by thin-layer chromatography of whole cell methanolysates on Silufol plates in a petroleum ether : diethyl ether (85 : 15) system. The plates were developed with a 5% solution of phosphomolybdic acid in alcohol.

Bacterial species were identified according to the procedures described in [19, 20].

Algal cultures were grown in a luminostat in batch conditions under constant illumination (1000 lx) by fluorescent lamps at 18–22°C. Microscopes Dokuval and Axiostar plus (Karl Zeiss Jena, GDR) equipped with phase contrast optics and a digital camera were used for microscopy.

In order to determine the effect of bacterially produced compounds on algal growth, pure bacterial cultures were grown on M9 medium with glucose to the late exponential or early stationary phase. The cells were then removed by centrifugation and the supernatant was filter-sterilized (Millipore, 0.2 μm). The culture liquid (CL) was then added to the algal medium (50%). In the control, the same amount of M9 sterile medium was added. Algal growth was monitored by chlorophyll content in the liquid medium or in the biomass washed off the plates with sterile medium. Pratt medium was also used for cultivating algae on plates. Algal biomass was collected by centrifugation. Chlorophyll was extracted with ethanol, and extinction at 649, 665, and 750 nm was determined. Chlorophyll concentration (Chl, μg/ml) was calculated using the equation:

$$Chl = (6.1\,(E_{665} - E_{750}) + 20.04\,(E_{649} - E_{750}))\,K, [21]$$

where E is extinction at the corresponding wavelength; K is the dilution factor; and 6.1 and 20.04 are extinction coefficients.

For combined cultivation of algae and bacteria in liquid media, mixed cultures were used. Pure cultures of algae (10 vol %) and one or more of the investigated bacteria (2–3 vol %), both collected at the stationary growth phase, were used to inoculate liquid M9 medium. Binary cultures were incubated similarly to algal cultures, i.e., at 18–22°C, under illumination, without shaking. For combined cultivation on agarized M9 and Pratt media, algae were streak-inoculated on one half of the petri dish, and bacteria on the other half; the plates were then incubated in a luminostat.

Bacterial Gram reaction was determined by staining [22].

All the experiments were carried out with 3–9 repetitions; the Student t test or the sign and Wilcoxon criteria were used to determine the reliability of the results by means of the Statistics 6.0 software package (StatSoft, Inc., United States). The absolute values characterizing algal growth varied significantly due to the high variability of algal growth and its seasonal fluctuations. The results of typical experiments are therefore presented on the illustrations.

RESULTS AND DISCUSSION

Characterization of the C. reinhardtii Dang. Culture

In the course of our experiments, some cells different from *C. reinhardii* Dang. typical vegetative cells were revealed. To identify these cells in batch culture, the life cycle of *C. reinhardii* was studied in hanging drop preparations. The haploid phase predominated in the life cycle; both sexual and asexual reproduction occurred. In the course of asexual reproduction, mitotic division of the vegetative mother cell results in formation of four daughter cells (zoospores) within its envelope. The zoospores, liberated after the rupture of the mother cell envelope, grow to the size of the mother cell and then divide. Sexual replication commences with mitotic division of the haploid mother cell. The daughter cells, liberated after the rupture of the mother cell envelope, do not increase in size, but conjugate immediately. Since the sexual process of *C. reinhardii* is isogamic, the terms “+" and “–” fertile individuals (copulative monadic isogametes) are used, rather than "male”

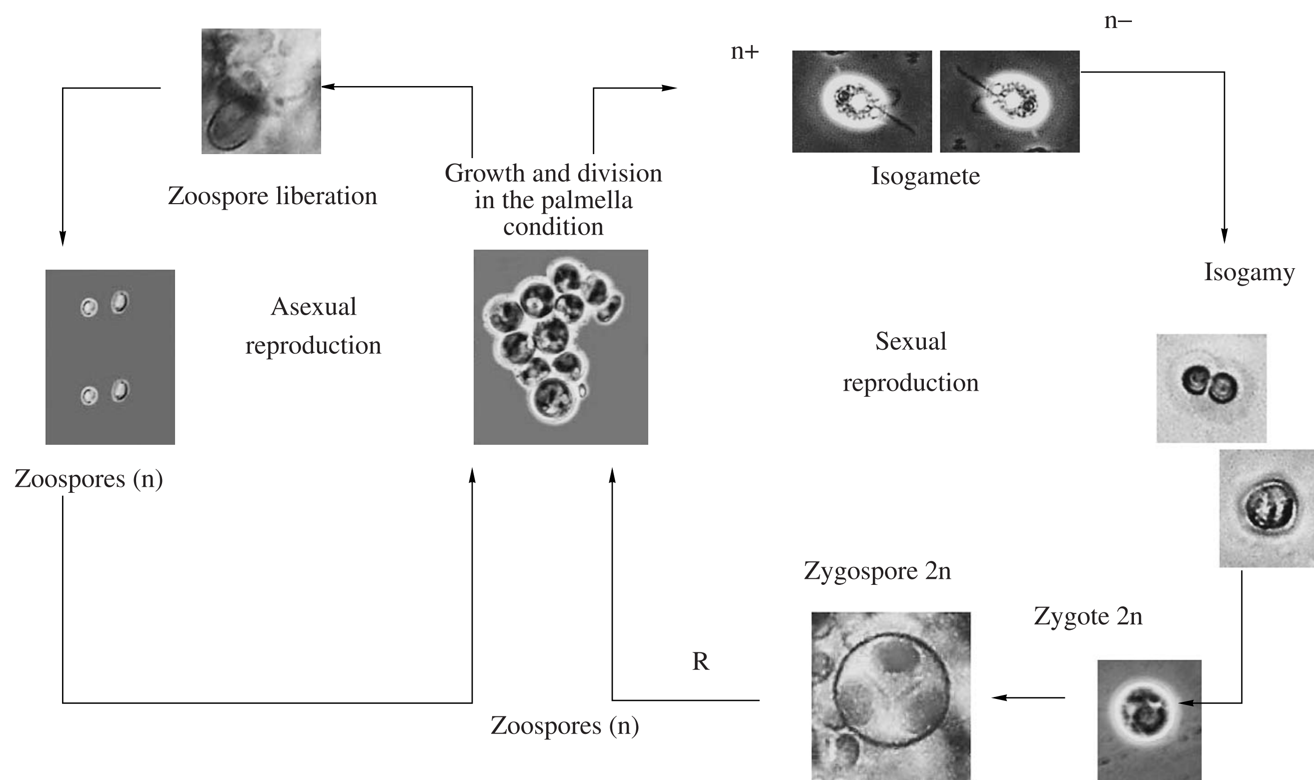

Fig. 1. Life cycle of *Chlamydomonas reinhardtii.*

and "female" gametes [11]. The conjugation results in formation of a zygote. The zygote can develop a strong envelope and thus survive unfavorable conditions as a zygospore. After a period of metabolic rest, the zygote undergoes meiotic division, resulting in the formation of four autospores. They become liberated from the zygospore envelope and grow to the size of the initial cell (Fig. 1).

The cells of unusual shape revealed by microscopy were therefore identified as chlamydomonas zygospores.

Under unfavorable conditions (exhaustion of the medium, accumulation of toxic metabolites, decreased temperature, or changes in the regime of illumination), motile cells can transform into a locomotory resting stage with granular cytoplasm (palmella condition); this condition can be reversed by optimization of environmental factors (Fig. 1).

Thus, *C. reinhardtii* Dang. cells are morphologically heterogeneous. This heterogeneity should be taken into account in works on the cultivation of this alga together with other microorganisms.

Isolation of Bacterial Satellites

Plating of the samples from an algologically pure *C. reinhardtii* culture on PYG and M9 with glucose revealed four colony types. Their color was pink, beige, yellow, and light pink; bacterial pure cultures were therefore designated P, B, Y, and P2, respectively. The morphological, physiological, and biochemical characterization of all the isolates was performed.

All the cultures were gram-positive. Their characteristics were as follows:

Yellow. Yellow or orange–yellow mucous colonies without aerial mycelium are formed; diffusing pigment is not produced. The isolate grows well on nutrient agar, modified Czapek medium, liquid Raymond medium with diesel fuel or paraffin, and on nutrient agar with 7% NaCl. Elongated rods predominate during the first 24 h after inoculation; shorter and fragmented rods, after 48–72 h. The fragmented short cells undergo binary division; the resulting rod-shaped cells are usually in V-like, Y-like, or palisade formations.

The cell wall of these organisms contains *meso*-diaminopimelic acid, arabinose, and galactose (type 4 cell wall), as well as mycolic acids. The bacteria are nonmotile facultative aerobes, amylase-, catalase-, and oxidase-positive; phosphatase-negative. Gelatinase and urease are produced. Nitrates are reduced to nitrites; tyrosine is decomposed. Metabolism is of the respiratory type. Acids are not produced from fructose, lactose, sucrose, maltose, mannitol, sorbitol, and galactose. Fructose, glucose, L-asparagine, citrate, or α-ketoglutarate can be used as the sole carbon source. Bacteria do not grow on media with monoethanolamine. The culture is sensitive to egg lysozyme (≥0.5 μg/ml),

gentamycin, neomycin, oleandomycin, lincomycin, cephazolin, levomycetin, and rifampicin; it is resistant to oxacillin and polymyxin. The strain survives prolonged storage on solid media under paraffin oil.

Beige. All the characteristics are identical to those of the "yellow" isolate, except colony color (beige or orange), capacity for fermentation, higher sensitivity to lysozyme (lysis occurred at its concentrations ≥0.25 μg/ml), and low survival when stored on solid media.

Based on their biochemical characteristics and the cell wall composition, strains B and Y were identified as *Rhodococcus terrae*.

Pink. The colonies are pink, small, mucous, and without aerial mycelium; diffusive pigment is not produced. The isolate grows well on nutrient agar. Short rods predominate during the first 24 h of cultivation; after 48–72 h they become shorter, fragmented, and form chains of three to seven cells. Bacteria are motile, facultative aerobes. Metabolism is of the fermentative type. Glucose, citrate, acetate, or lactate can be used as sole carbon sources. The strain does not survive well on solid media.

Based on their biochemical characteristics and the cell wall composition, strain P was assigned to the genus *Bacillus*.

In the course of storage, each of the strains Y, B, and P dissociates into two variants with different pigmentation and nutrient requirements; they were not studied in detail. When cultivated together with the alga on solid media, these strains stimulated algal growth.

Light pink. Small pale pink convex colonies, mucous, with an even edge, without aerial mycelium are formed; diffusive pigment is not produced. The cells are gram-positive cocci. After 48 h, they increase in size and divide; the cells are located individually or in groups of two or four. They are nonmotile aerobic bacteria, catalase- and oxidase-positive, with respiratory metabolism. Acid is produced from glucose, lactose, fructose, maltose, and galactose; sucrose is not fermented. The isolate grows on nutrient agar with 7% NaCl. Mycolic acids and *meso*-DAPA were not detected in the cell wall; lysine and ornithine were the only amino acids revealed.

The isolate is sensitive to oleandomycin, lincomycin, oxacillin, polymyxin, cephazolin, levomycetin, rifampicin, and gentamycin; it is resistant to neomycin.

Based on their morphological and biochemical characteristics, the isolate was identified as *Micrococcus roseus*.

Apart from these four strains, the slowly growing strain O (oligotrophic) was repeatedly isolated from the association. It could grow only on Pratt medium without carbon sources and on M9 medium with acetate. It has never been isolated on media with peptone and glucose. The colonies appeared after 7–9 days of incubation. The cells of strain O are gram-positive spore-forming rods. No algal growth occurred on solid media in the presence of this strain.

Growth Physiology of C. reinhardtii Pure Culture, Its Native Association with Bacteria, and Associations with Bacterial Isolates

The pure and associated cultures of *C. reinhardtii* differed in several aspects.

While the pure culture required monthly transfers, the algae in the native association survived longer (up to several months). In liquid pure cultures, microscopy revealed mostly nonmotile cells and a number of lysed, involutory (with granular cytoplasm), and palmella-state cells after two weeks of incubation. In association with bacteria, all the algal cells were motile; lysed and resting cells were practically absent. In the case of exhausting streak inoculation on agarized Pratt medium, single algal colonies in pure culture appeared later (7th–10th day of incubation) and were smaller (not more than 1 mm) than in the presence of bacteria (5th–6th day and 2–4 mm, respectively).

Bacterial satellites were therefore assumed to stimulate *C. reinhardtii* growth; this hypothesis was confirmed experimentally.

When the pure culture of *C. reinhardtii* was streak-inoculated on one half of a petri dish, its growth was better in the presence of bacterial satellites (strains P, Y, and B) on the other half of the dish than in the case of bacteria-free culture.

The growth curves of *C. reinhardtii* in pure culture, in the native association, and in binary or mixed cultures with strains B, Y, and P (*Rhodococcus terrae* and *Bacillus* sp.) in liquid M9 medium are presented on Fig. 2.

The native association of algae and bacteria had the highest growth rate. Introduction of bacterial pure cultures also stimulated algal growth; the beige and yellow strains had a more pronounced effect. In the binary culture with strain P, the period of pronounced growth stimulation was followed by the lysis of the algal culture. In some cases strain P inhibited algal growth throughout the experiment (data not shown). In mixed cultures with *Rh. terrae* strains Y and B, the maximal specific growth rate of the alga increased from 0.4 to 0.6–0.7 day^{-1}; this value is not much less than the growth rate of the native association (0.76 day^{-1}). Chlorophyll synthesis in mixed cultures increased from 10 to almost 30 μg Chl/ml of the culture, while in the native association it was still much higher (up to 60 μg/ml).

Mechanism of Bacterial Effect of the Alga

Stimulation of algal growth by bacterial satellites was attributed to several factors [7–9], including CO_2 production, oxygen consumption, and production of growth stimulators. Our results suggest the effect of

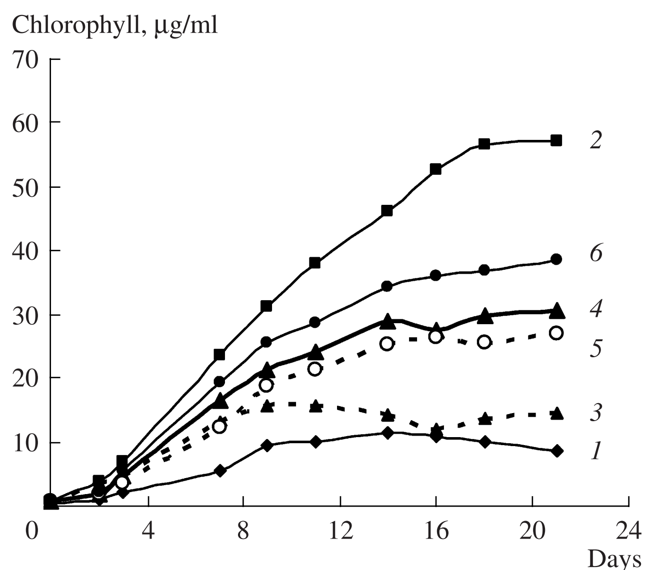

Fig. 2. Growth of *C. reinhardtii* in liquid cultures in association with satellite strains and in the native association: pure culture (*1*); native association (*2*); together with strain P (*Bacillus* sp.) (*3*); together with strain B (*Rh. terrea*) (*4*); together with strain Y (*Rh. terrae*) (*5*); together with strains B, Y, and P (*Bacillus* sp. and *Rh. terrae*) (*6*).

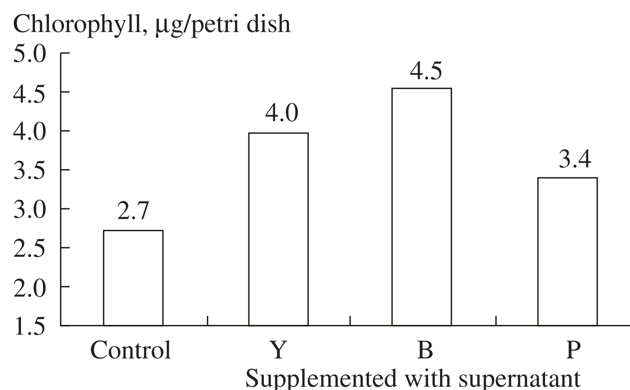

Fig. 3. The yield of *C. reinhardtii* on agarized Pratt medium supplemented with the supernatants of bacterial satellite strains incubated in distilled water. Distilled water was added in the control series.

growth stimulators. In our experiments, culture liquids of bacteria were added to the medium for algal cultivation. Increased yield (chlorophyll biosynthesis) on agarized media was used as an indication of the presence of growth stimulators. The effect of supernatants of the cell suspensions of strains Y, B, and P, incubated in distilled water for 24 h (i.e., under stress conditions, inducing autolysis of bacterial cells) was also determined. After 4–6 days of incubation, the yield was 25–66% higher in the presence of the supernatants (Fig. 3). The supernatant of the beige strain had the most pronounced stimulatory effect.

In the case of the culture liquid of bacteria grown under optimal conditions (on M9 medium with glucose), the effect was quite different. For example, CL of strain Y grown under these conditions until the early stationary growth phase did not stimulate algal growth and in some cases even decreased its yield by 30–50% (from 1.6 to 1.1–0.8 μg chlorophyll per petri dish after 4–6 days of cultivation). Accumulation of alkylhydroxybenzenes (AHB) in the CL is among the possible explanations for its inhibitory effect on algal growth, similar to the previous findings for a number of bacteria [24, 25].

Thus, the cultures of bacterial satellites can contain two types of metabolites with an opposite effect; this is evidently an adaptive device. In the absence of nutrient sources (i.e., under conditions promoting bacterial autolysis), compounds stimulating algal growth are released into the medium; enhanced development of algae supports the recovery of the surviving bacteria. Under the growth conditions favorable for bacteria, stimulants are not excreted. Moreover, inhibitors of algal growth are produced, since chlamydomonads can compete with bacteria for carbon sources.

The finding that an algo–bacterial association grows better (has a higher growth rate, cell density, and chlorophyll yield) and survives longer than a pure algal culture is of considerable ecophysiological interest. Since bacteria receive organic compounds that they cannot synthesize themselves, they gain self-evident advantages from this cooperation. Bacteria, in turn, promote algal development by synthesizing growth-stimulating compounds, which are liberated into the environment in the case of bacterial autolysis. In the presence of carbon sources, bacteria can also suppress algal growth.

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REFERENCES

1. Doucette, G.J., Interactions between Bacteria and Harmful Algae: a Review, *Nat Toxins*, 1995, vol. 3, no. 2, pp. 65–74.
2. Glagoleva, O.B., Zenova, G.M., and Dobrovol'skaya, T.G., Intreaction of Algae with Bacterial Satellites in Associative Cultures, *Algologiya*, 1992, vol. 2, no. 2, pp. 57–63.
3. Maksimova, I.V., Interactions of Algae with Bacteria and Other Microorganisms in Mixed Cultures, *Biologiya avtotrofnykh mikroorganizmov* (Biology of Autotrophic Microorganisms), Moscow: Mosk. Gos. Univ., 1966.
4. Mayali, X. and Azam, F., Algicidal Bacteria in the Sea and Their Impact on Algal Blooms, *J. Eukaryot. Microbiol.*, 2004, vol. 51, no. 2, pp. 139–144.
5. Bauer, W.D. and Robinson, J.B., Disruption of Bacterial Quorum Sensing by Other Organisms, *Curr. Opin. Biotechnol.*, 2002, vol. 13, no. 3, pp. 234–237.

6. Potin, P., Bouarab, K., Kupper, F., and Kloareg, B., Oligosaccharide Recognition Signals and Defence Reactions in Marine Plant-Microbe Interactions, *Curr. Opin. Microbiol.*, 1999, vol. 2, no. 3, pp. 276–283.
7. Vladimirova, M.G. and Bazaitova, L.V., Development of *Chlorella pyrenoidosa* and the *Pseudomonas oleovorans* Group Bacteria in Mixed Culture, *Mikrobiologiya*, 1961, vol. 30, no. 4, pp. 593–598.
8. Parker, B.C. and Bold, H.C., Biotic Relationships between Soil Algae and Other Microorganisms, *Am. J. Bot.*, 1961, vol. 48, no. 2, pp. 185–190.
9. Bunt, J.I., Blue-Green Algal Growth, *Nature*, 1961, vol. 192, pp. 1274–1278.
10. Topachevskii, A.V. and Masyuk, N.P., *Presnovodnye vodorosli Ukrainskoi SSR* (Freshwater Algae of the Ukrainian SSR), Kiev: Vishcha Shkola, 1984.
11. Gorbunova, N.P., *Al'gologiya: ucheb. posobie dlya VUZov.* (Algology: a Textbook), Moscow: Vysshaya shkola, 1991.
12. Vasser, S.P., Kondrat'eva, N.V., Masyuk, N.P., Palamar'-Mordvintseva, G.M., Vetrova, Z.I., Kordyum, E.L., Moshkova, N.A., Prikhod'kova, L.P., Kovalenko, O.V., Stupina, V.V., Tsarenko, P.M., Yunger, V.P., Radchenko, O.V., Vinogradova, O.N., Bukhtiyarova, L.N., and Razumna, L.F., *Vodorosli. Spravochnik* (Algae: a Handbook), Kiev: Naukova Dumka, 1989.
13. Dedusenko-Shchegoleva, N.T., Matvienko, A.M., and Shkorbatov, L.A., *Opredelitel' presnovodnykh vodoroslei SSSR. Vyp. 8. Zelenye vodorosli.* (Freshwater Algae of the USSR: Igentification Guide. Iss. 8. Green Alggae), Moscow, Leningrad, Akad. Nauk SSSR, 1959.
14. Harris, E.H., *The Chlamydomonas Sourcebook*, San Diego: CA, USA: Academic, 1989.
15. Egorov, N.S., *Praktikum po mikrobiologii* (A Practical Course on Microbiology), Moscow: Mosk. Gos. Univ., 1976.
16. Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning, 2nd ed.* Cold Spring Harbor Lab. Press, 1989.
17. Navashin, S.M. and Fomina, I.P., *Ratsional'naya antibiotikoterapiya (spravochnik)* (Rational Antibiotics Therapy: a Handbook), Moscow: Meditsina, 1982.
18. Zvyagintsev, D.G., (Ed.) *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Moscow: Mosk. Gos. Univ., 1991.
19. *The Shorter Bergey's Manual of Determinative Bacteriology*, Holt, J.G., Ed., Baltimore: Williams & Wilkins, 1977. [Russ. Transl.: *Kratkii opredelitel' bakterii Bergi*, Moscow: Mir, 1980].
20. Nesterenko, O.A., Kvasnikov, E.L., and Nogina, T.M., *Nokardiopodobnye i korinepodobnye bakterii* (Nocardiaform and Coryneform Bacteria), Kiev: Naukova Dumka, 1985.
21. Lichtenthaler, H., E. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes, *Methods Enzymol.*, 1987, vol. 148, pp. 350–382.
22. *Manual of Methods for General Bacteriology*, Gerhardt, P. et al., Eds., Washington: Amer. Soc. Microbiol., 1918 [Russ. Transl. Moscow: Mir, 1984].
23. Hollander, M. and Wolfe, D.A., *Nonparamertic Statistical Methods*, N-Y, L: Sydney, Toronto: John Willey and Sons, 1973.
24. Mulyukin, A.L., Kozlova, A.N., Kaprel'yants, A.S., and El'-Registan, G.I., The d_1 Autoregulatory Factor in *Micrococcus luteus* Cells and Culture Liquid: Detection and Accumulation Dynamics, *Mikrobiologiya*, 1996, vol. 65, no. 1, pp. 20–25 [*MIcrobiology* (Engl. Transl.), vol. 65, no. 1, pp. 15–21].
25. Nikolaev, Yu.A., Mulyukin, A.L., Stepanenko, I.Yu., and El'-Registan, G.I., Autoregulation of Stress Response in Microorganisms, *Mikrobiologiya*, 2006, vol. 75, no. 4, pp. 489–496 [*MIcrobiology* (Engl. Transl.), vol. 75, no. 4, pp. 420–426].