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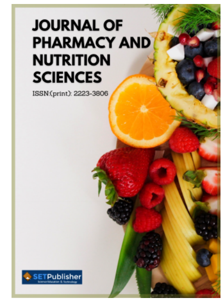

Impact of Biogenic Amines on the Growth of a *Chlorella vulgaris* Culture

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Abstract:

The present work aims to develop a new approach enabling biotechnologists to increase the yield of *Chlorella vulgaris* biomass by means of biogenic amines (serotonin, dopamine, and histamine) that are known to stimulate growth of various unicellular organisms. *C. vulgaris* strain ALP was cultivated in the light with constant aeration at 24°C in a minerals-containing medium. Experimental systems contained 1, 10, or 100 μM of dopamine, histamine, or serotonin. Algal cells were counted using a light microscope. Serotonin caused a slight increase in biomass yield at a concentration of 10 μM, but not at the other tested concentrations. 1 and 10 μM (but not 100 μM) dopamine increased the cell number in the *C. vulgaris* culture at early cultivation stages. Histamine is the most efficient growth stimulator at concentrations of 1 and 10 μM, but not at a concentration of 100 μM, which even proved inhibitory to the algal culture. The data obtained demonstrate that the neurochemicals exert a stimulatory influence on the growth of the *Chlorella* culture at relatively low (micromolar) concentrations. Since animals often produce biogenic amines in response to stress or injury, the data give grounds for the suggestion that planktonic algae can benefit, in terms of growth rate, from the substances released by stressed or wounded representatives of aquatic fauna. In biotechnological terms, the data obtained hold some promise with regard to developing a relatively economical technique of boosting *Chlorella* biomass production.

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INTRODUCTION

The green unicellular alga chlorella (with *Chlorella vulgaris* as a typical species) forms a part of the natural phytoplankton of freshwater ecosystems. It plays an important role in the trophic networks of these ecosystems; for instance, it serves as food for daphnia and other aquatic invertebrates [1, 2]. Apart from its ecological function, chlorella can be used as (i) a food additive for preparing bread, pasta, and ice cream, etc.; (ii) a drug; and (iii) a novel kind of biofuel [3-5]. This alga is of potential practical interest as a food source to be used in future space voyages [6].

In light of the above, developments aimed at increasing chlorella biomass yield are of indisputable interest. In the literature, forced culture aeration and stirring are considered among the possible methods of attaining this goal [3]. Besides, much attention has been given to the use of growth-accelerating agents such as, e.g., sodium selenite [3, 7].

Biogenic amines belong to neurochemicals (neuromediators or neurotransmitters) that transfer impulses from neuron to neuron or between the cells of sensory organs and nerve cells. A large number of neurochemicals function as signals and effectors in various kingdoms of life, including plants [8-13], prokaryotic and eukaryotic microorganisms [10, 13-16]. Although research work exploring the effects of neurochemicals on some algal species has already been conducted (see next paragraph), their impact on chlorella is still unexplored. Therefore, in this work, we have tested some biogenic amines for their capacity to stimulate/accelerate the growth of *Chlorella vulgaris.*

It has been established that green algae belonging to the phyla *Charophyta* and *Chlorophyta* include species that synthesize neurochemicals or neurochemicals-binding receptors [9-11]. The most widely spread neuro-chemical in the realm of algae is histamine, although some species also contain acetylcholine that is used by *Charophyta* cells for electrical signal transmission, in an analogy to animal neurons [9-11, 17]. The species *Chara australis* was found to produce serotonin, whose concentrations undergo diurnal oscillations. Serotonin is envisaged as the precursor of melatonin that presumably is involved in regulating circadian rhythms in *C. australis* [17]. *Ulvaria obscura* releases dopamine as a herbivore defense mechanism [17].

The goal of the present work was to test the growth effects of the biogenic amines serotonin, dopamine, and histamine on the culture of *Chlorella vulgaris* ALP.

MATERIALS AND METHODS

C. vulgaris strain ALP was cultivated in the light with constant aeration at 24°C in the medium used in [2] with the following composition (g/L) : KNO_3 2.5; $MgSO_4$ x 7H2O 1.25; $KH_2PO_4$0.625; $FeSO_4$ x $7H_2O$ 0.003; ethylene diamine tetraacetate 0.185; microelement mixture 1 mL per 1 L (pH 6.0); the microelement mixture composition was as follows (g/L): H_3BO_3: 2.86; $MnCl_2$ x $4H_2O$: 1.81; $ZnSO_4$ x $7H_2O$: 0.222; MoO_3 176.4 mg/10 L; NH_4VO_3: 229.6 mg/10 L; $CuSO_4$ x $5H_2O$: 0.01 mg/L; $Co(NO_3)_2$ x $4H_2O$: 0.146; KJ:0.083; $NaWO_4$ x H_2O: 0.033; $NiSO_4(NH_4)SO_4$ x $6H_2O$: 0.198. An actively growing culture was used as inoculum and diluted by the medium to a final cell concentration of $1.4x10^6$ cells/cm^3, which corresponded to an optical density (OD) of 0.12±0.1 at λ = 540 nm. The culture was grown until the OD value reached a plateau level.

Experimental systems contained 1, 10, or 100 μM of dopamine hydrochloride, histamine hydrochloride, or serotonin hydrochloride that were added to the inoculum as freshly prepared aqueous solutions; the control system was supplemented with an equal volume of water at inoculation. All neurochemicals were of analytic grade, purchased from the Sigma company (USA). Algal cells were counted using a light microscope (in its fields of view), and their number was calculated for a culture volume of 1 mL. In some experiments, we used a calibration curve in order to estimate the cell number in the cultures based on the optical density values at 540 nm. OD values were measured using a LOMO spectrophotometer (Russia). 4-5 independent repeats of each experiment were performed; the results were statistically treated, and the mean values and the standard deviations were calculated.

RESULTS AND DISCUSSION

The growth dynamics of *Chlorella vulgaris* strain ALP cultures in the presence of the biogenic amines dopamine, histamine, and serotonin, or without them (control) is shown in Tables **1**, **2**, and **3**. The selected time points (1, 2, 3, and 4 days of cultivation) corresponded to the lag phase, the early exponential phase, the late exponential phase, and the stationary phase, respectively.

Serotonin

This derivative of the amino acid tryptophan, a major brain neurotransmitter and a histohormone produced

Table 1: Growth dynamics of *C. vulgaris* cultures (calculated as cell numbers per 1 mLx10^{-6}) cultivated with or without the biogenic amine serotonin. Note: The Table contains the averaged results of 4-5 repeats; standard deviations are given

Days of cultivation	Control	1 μM serotonin	10 μM serotonin	100μMserotonin
0 (inoculation)	1.4	1.4	1.4	1.4
1	7.6±0.6	7.5±0.6	8.8±0.6	7.7±0.6
2	14.6±0.8	14.3±0.9	16.2±0.9	16.2±0.9
3	27.2±1.0	26.7±0.9	28.6±0.9	25.74±0.8
4	28.6±1.0	28.5±1.0	31.0±1.1	28.6±1.0

Table 2: Growth dynamics of *C. vulgaris* cultures (calculated as cell numbers per 1 mLx10^{-6}) cultivated with or without the biogenic amine dopamine. Note: The Table contains the averaged results of 4-5 repeats; standard deviations are given

Days of cultivation	Control	1 μM dopamine	10 μM dopamine	100 μM dopamine
0 (inoculation)	1.4	1.4	1.4	1.4
1	7.7±0.6	12.2±0.8	11.4±0.7	9.0±0.6
2	14.5±0.8	20.6±0.9	18.2±0.9	16.3±0.8
3	26.9±1.0	30.8±1.1	29.3±1.0	28.6±1.0
4	28.3±1.0	31.0±1.0	30.0±1.1	29.8±1.0

along with histamine in response to inflammation, produced no statistically significant effect on the growth of the *C. vulgaris* culture at the lowest tested concentration, 1 μM. However, the medium concentration of serotonin, 10 μM, caused a slight (≈ 12%) but statistically verifiable increase in biomass yield after four days of cultivation. This marginal stimulation was not observed with the highest tested serotonin concentration (100 μM). Towards the end of the cultivation period (after four days), precipitation of a significant part of *C. vulgaris* biomass was documented. This pointed to a toxic effect of serotonin at high concentrations.

Dopamine

This neurotransmitter and lactation-inhibiting hormone in mammals produced from tyrosine markedly (by over 50%) increased the cell number in the *C. vulgaris* culture at early cultivation stages (1-2 days). However, this increase became less significant at later cultivation stages, suggestive of a growth-acceleration rather than stimulatory action on the culture. This effect was characteristic of 1 and 10 μM dopamine, but the growth increase was not observed at the highest tested concentration (100 μM); presumably, another inhibitory effect caused by this dopamine concentration overrode the accelerating effect.

Histamine

This derivative of the amino acid histidine proved to be the most efficient growth stimulator at the low and the medium concentration (1 and 10 μM, respectively). The increase in biomass already manifested itself at the early growth stages, but it was more significant towards

Table 3: Growth dynamics of *C. vulgaris* cultures (calculated as cell numbers per 1 mLx10^{-6}) cultivated with or without the biogenic amine histamine. Note: The Table contains the averaged results of 4-5 repeats; standard deviations are given

Days of cultivation	Control	1 μM histamine	10 μM histamine	100 μM histamine
0 (inoculation)	1.4	1.4	1.4	1.4
1	7.8±0.6	13.4±0.9	14.3±0.9	8.6±0.7
2	14.2±0.8	19.7±1.0	22.6±1.1	13.9±0.8
3	27.5±1.0	38.3±1.4	49.33±1.5	26.1±1.0
4	28.6±1.1	47.2±1.5	57.2±1.6	11.3±0.9

the end of the cultivation period (day 4), amounting to ≈100% of the control value. Like dopamine, histamine failed to produce its stimulatory effect when applied at the highest (100 μM) concentration; moreover, it exerted an inhibitory influence on the culture's growth towards the end of its cultivation.

The data obtained in this work demonstrate a stimulatory effect of biogenic amines on the growth of the *C. vulgaris* culture. In an analogy to animal and bacterial systems, these data seem to suggest a specific, presumably membrane receptor-dependent, mode of action of the aforementioned biogenic amines. However, a decrease in amplitude of the growth-accelerating/stimulating effect was observed with a comparatively high concentration (100 μM) of the amines, with 100 μM histamine even causing growth suppression. This can be explained by assuming an additional nonspecific inhibitory effect that can result, e.g., from decoupling the cell membranes. At high concentrations, serotonin has previously been reported to decrease the membrane potential in the cells of the purple phototrophic bacterium *Rhodospirillum rubrum* [18].

Hence, these data can be interpreted in terms of the hypothesis that *C. vulgaris* cells contain specific biogenic amines-binding receptors, in an analogy to the bacterial species where serotonin behaves as a quorum-sensing signal that enables bacteria to estimate their population density and to modify their behaviors accordingly. In *Pseudomonas aeruginosa*, serotonin functions as the signal in the quorum-sensing system *lasI*-*lasR*. An increase in its concentration results in increasing *Ps. aeruginosa* virulence and biofilm formation both *in vitro* and in the organism of an infected mouse [19].

The data seem to have interesting implications for the ecological situation in water bodies that represent the natural habitats of *C. vulgaris.* Dopamine is one of the catecholamines released by animal organisms under stress, causing the stressed animal to produce also norepinephrine (noradrenaline) and epinephrine (adrenaline). Inflamed or injured tissues liberate serotonin and, more so, histamine. Both fish and aquatic invertebrates (zooplankton) are known to produce all the three aforementioned neurochemicals in water ecosystems that also include phytoplankton, which is exemplified, in this work, by *Chlorella.* The implication is that stress factors or various injuries sustained by aquatic animals are expected to accelerate algae growth like *C. vulgaris* and increase its biomass yield. The reason is, to reiterate, that an injured or stressed animal organism, whether a fish or, e.g., a crustacean, should release biogenic amines that will produce their stimulatory effect on the algae. In a similar fashion, human gut bacteria grow faster in the presence of neurotransmitters whose production and liberation into the lumen are facilitated by local inflammation, i.e., the gut microbiota is "interested" in keeping the intestine in a locally inflamed state (reviewed, [13]).

CONCLUSION

The data obtained in this work demonstrate that the neurochemicals tested by us exert some stimulatory influence on the growth of the *Chlorella* culture at relatively low (micromolar) concentrations.

In biotechnological terms, the data obtained hold some promise with regard to developing a relatively economical technique of boosting *Chlorella* biomass production in an industrial setting. This area of research may be of potentially practical interest concerning future space journeys (e.g., to Mars) because vitamin-rich *Chlorella* biomass is likely to be one of the staple items in the astronauts' diet [6]. With sunlight being the only available energy source in space, the cultivation of photosynthesizing organisms such as algae seems to be a feasible and economical project on board a space ship. The idea of testing the growth effects of neurochemicals on unicellular algae was inspired by literature data (partly quoted above) on the promotion of the growth of diverse microbial species by the addition of low (micromolar) concentrations of neuroactive substances such as biogenic amines. Identifying a growth-stimulating neurochemical that is active at these low concentrations would enable using it during a long-term space voyage to increase the algal biomass yield. The whole procedure would probably be quite economical, given the negligible price of the insignificant amounts of the neurochemicals involved.

CONSENT FOR PUBLICATION

The authors confirm that all experiments with algae were performed in accordance with the national and international standards and guidelines. This article does not contain any studies involving animals performed by any of the authors.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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