Research Article

The Effect of Gibberellic Acid on the Production Characteristics and Biochemical Parameters of Tetraselmis Suecica in an Enrichment Culture

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Abstract. The use of gibberellic acid as a stimulator of microalgae growth has beensubstantiatedexperimentally.This research aimed to assess the effect of exposure to a wide range of gibberellic acid concentrations on the growth dynamics ofthe microalga*Tetraselmissuecica*in an enrichment culture. The duration of the experiments was 14 days. It has been shown that gibberellic acid,atconcentrations of 0.39–3.20× 10^{-8}M, stimulates algaegrowth. In this research, the exposure to gibberellic acid at concentrations of 0.39–3.20 × 10^{-8}M was accompanied by a variation in the pattern of growth curves: the maximum number of cells was recorded on day seven of the experiment. A higher concentration of the phytohormone (3.84 × 10^{-8}M) inhibited the increase inculture density. The growth of the*T. suecica*culture in the control group was 332%;the growth of the culture exposed to gibberellic acid at a concentration of 0.39 × 10^{-8}M was1136%. The values of the specific growth rate of*T. suecica*were estimated for different periods of cultivation. On day14 of the experiment, the biochemical composition of microalgae biomass was analyzed.According to the results, gibberellic acid stimulated the accumulation of carbohydrates, proteins, and chlorophyll. Nevertheless, the phytohormone had no effect on lipidaccumulation. An assumption was made thatexposure to low concentrations of phytohormone stimulates the growth of microalgae by reducing the lag phase of growth.

Keywords: gibberellic acid, microalga, cultivation, lipids, carbohydrates, proteins

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1. Introduction

Microalgae are photosynthetic microorganisms widely distributed in various natural habitats. The high growth rates characteristic of microalgae provides the economic efficiency of their cultivation. Currently, almost 25 species of microalgae are commercially cultivated. Despite numerous studies on nutritional value of hundreds of microalgae species, only few of them are grown in aquaculture.

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*Tetraselmissuecica*is used in aquaculturesmostlyas livefeed for cultured invertebrates such as bivalve mollusks (oysters andscallops)andsea cucumbers (Conceiçãoet al., 2010; Camacho-Rodríguezet al., 2014; Chautonet al., 2015).

Microalgae used as live feed are sensitive to the composition of culturemedium and cultivation conditions and differ in their biochemical structure. The promising research conducted in this fieldisofmajorscientific and practical significance: the use of microalgae cultures contributes to the increase in weight, survival rate, and nutritional value of animalsgrown in mariculture. Furthermore, knowledge of the physiological effects of phytohormones opens upa wide range of opportunities for their industrial use in mariculture farms.

Descriptions of the composition of phytohormones and the hormone-like action of extracts from microalgae are scarce and concern mainly freshwater species. Information on microalgae phytohormones is summarized in the works of E.A. Romanenko and co-authors (2015; 2016).

We could not find available information about the use of phytohormones for the cultivation of microalgae, which are natural food for cultured animals. However, the high demand for 'live' starter feeds to grow outmollusks and other invertebrates necessitatesascientificsearchfor the feed production biotechnologies based on various microalgae species.

Data on the presence of gibberellins in marine microalgae are fragmentary. Gibberellic acid (GA) has been found in 31 microalgae species (Stirk et al., 2013). A high level of gibberellin-like substances has been recorded from*Tetraselmis* sp. (Chlorophyta) (Mowat, 1965).

Species belonging to different phyla of algae have different concentrations of exogenous GA which can have a pronounced stimulating effect (Pan et al., 2008; Park et al., 2013), or an inhibitory effect on the growth and size of dry biomass (Johnston, 1963; Bentley-Mowat, Reid, 1969),or no effectat all (Evans, Sorokin, 1971).

The regulatory action of gibberellins (GA) has been well studied on higher plants, but data on the possible effect of these hormones on the growth of algae is still insufficient. Exogenous gibberellins significantly reduce the lag phase and stimulate cell division and growth in the exponential phase of microalgae growth, increase total biomass values, contribute to accumulation of protein, chlorophylls, and carotenoids, and alsosignificantly attenuate toxic effects of heavy metals in algae habitat (Romanenko et al., 2016).

The goal of our study was to assess the effect of exposure to exogenous GA on the growth and biochemical parameters of *Tetraselmissuecica*in an enrichment culture.

2. Materials and methods

As a source material for cultivation, a laboratory-grown,algologically pure culture of *Tetraselmissuecica* from the collection of the Far Eastern State Technical Fisheries University (Dalrybvtuz) was used. Heat-resistant conical 1 Lglass flasks were used for cultivation. Microalgae were grown in theenrichmentmode on the Goldberg's nutrient medium (Kabanova, 1961). The microalgae culture was kept under a temperature of 21–23°C, an illuminance level of 8–10 lx, a light : dark cycle of 8: 16 h, and with stirring (agitation) four or five times a day.

GA (Hebei Guanlang Biotechnology Co., Ltd, China)was used as a phytohormone stimulating growth.

The microalga was cultivatedas a monoculture. Increase in algae biomass was detected asanincreaseinthe number of cells counted in each experiment in three Goryaev chambers under a light microscope. The duration of the experiments was 14 days.

Specific growth rate of microalgae was estimated according to R.P. Trenkensh (2019).

The total carbohydrate contentwasdetermined by the formation ofa colored green compound with a maximum absorbance at 625 nm as a result of reaction of 5-hydroxymethylfurfural, produced during glucose hydrolysis in a hot acidic medium, with theanthronereagent(Laurens etal., 2012).

Samples for measuringproteinwere prepared according toHerberwith co-authors (Herber etal., 1971).Protein content was measuredby the Lowry's method (Lowry etal., 1951).

Total chlorophylls wereobtained by acetone extraction from pre-frozen algae biomass (Carneiroetal., 2019). Contents of chlorophyllswerequantified spectrophotometrically at wavelengths 630, 647, 664, and 750 nm. As a control,90% acetone was used (Aminote-tal., 2001).

Total lipids were extracted by the Folch's method (Christie, 2003).Amount of lipids in microalgae was determined gravimetrically.

3. Results and discussion

Efficiency of microalgae cultivation is determined by the culture density and the rate of cell growth during experiment. As the results of our 14-day experiment show, the cell culture density in the control group increased from 0.3 to 1.77 million cells/mL, which

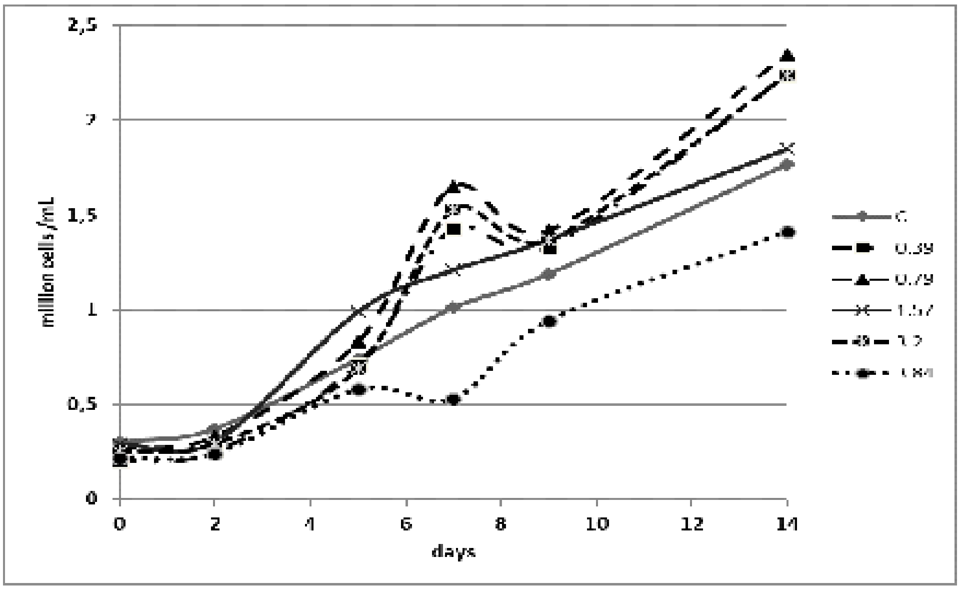

Figure 1: Effectofexposure to differentconcentrationsofgibberellic acid (0.39–3.84 $\times 10^{-8}$M; C - control) on the growth ofthe*Tetraselmissuecica*culture.

corresponds to a growth rate of105,000cells/day (Fig.1). The curve of cell culture growth for the control group had a linear/logarithmic shape.

The study of the exposure to different GAconcentrationson the growth of*T. suecica*revealed a dose-dependent effect. Thus, the phytohormone atconcentrations of 0.39–3.20$\times 10^{-8}$M stimulated the increase in number of cells;ata concentration of3.84$\times 10^{-8}$M, it inhibited the increase in cell number in the culture.

It should be noted that theexposure to GAat concentrations of 0.39–3.20$\times 10^{--8}$ Mcaused the growth curves of*T. suecica*to form a peak on day 7 of cultivation. Apparently, this can beexplained by the change of microalgaegenerations, as on the followingdaysthe number of cells in the culturereduced. A different pattern of the growth curve was observed in the case of exposure to the phytohormone at a concentration of3.84$\times 10^{--8}$M: on day 7 of cultivation, the number of cells in the culturealso decreased.

For all the phytohormone concentrations studied, the growth of the cell culture within the period of 7–14 days was linear.

Our findings are consistent with the results reported by Park et al. (2013), who showed that exogenous gibberellins significantly reduce the lag phase and stimulate cell division during the exponential phase of microalgae growth.

The specific growth rate during the logarithmic phase (on days 0–7 and 9–14 of cultivation) was estimated for all the experimental groups (Table 1).

TABLE 1: Effect of exposure to different gibberellic acid (GA) concentrations on the specific growth rate (days^{-1}) of *Tetraselmissuecica*.

Concentration of GA, 10^{-8}M	Cultivation period, days		Culture growth, %
	0–7	9–14	
Control	0.13		589
0.39	0.28	0.087	1136
0.79	0.26	0.076	882
1.57	0.25	0.051	639
3.2	0.26	0.082	897
3.84	0.2*	0.067	648
*For the period of 0–5days.			

An analysis of data in Table 1 shows that GA stimulated the culture growth more effectively during the first 7 days of cultivation. On days 9–14 of cultivation, the rate of specific growth of the culture decreased 3.2–4.9-fold.

To assess the effectiveness of phytohormone action, the daily increase in the number of cells was estimatedthroughout the experimental period. The initial number of cells in the culture was assumed to be 100% (Table 1).

It should be noted that the pattern of growth curves in the case ofGAexposuregenerally resembled the relationship of the culture growth rate in the control. However, the estimation of daily increase showed the most effective growth-stimulating GAconcentrationto be 0.39× 10^{-8}M. When exposed to this concentration, the culture growth compared to the initial value was 1136% for the entire period of experiment. With the other phytohormoneconcentrations, the growth stimulation amounted to 648–897%. In the control group, the increase in cell culture was 689%for the entire period of experiment.

Thus, the experiment provided promising results of GA exposure at a concentration of0.39 × 10^{-8}M to increase the efficiency of*T. suecica*cultivation in an enrichment culture.

Previously, it was reported that the physiological effects of GAon *Chlamydomonasreinhardtii* aremanifestedas the accumulation of protein and chlorophyll during the exponential growth phase (Park et al., 2013). Furthermore, high concentrations of the stimulatorcaused the carbohydrate contenttodecrease (Pan et al., 2008).

The biochemical composition of cells was determined in a freeze-dried culture exposed to a phytohormone concentration of 0.39 × 10^{--8}M (Table 2).

According to the data provided in Table 2,a GA exposure at a concentration of0.39 × 10^{--8}M had a weak stimulating effect on the accumulation of protein and carbohydrates

TABLE 2: Chemical composition of a freeze-dried culture of *Tetraselmissuecica*

Culture	Carbohydrates, %	Protein, %	Lipids, %	Chlorophyll, pg/cell	Ash, %
Control	32.4	38.7	5.7	5.2	4.7
Gibberellic acid (0.39 $\times$ 10^{--8}M)	36.0	40.8	5.1	5.9	4.8

in the culture. The stimulating effect on the accumulation of chlorophyll was approximately 13%. This concentration of the stimulator had almost no effect on the levels of lipids and ash in dry microalgae biomass.

Thus, the study has revealed a dose-dependent stimulation of growth of *T. suecica* in the enrichment culture. It has also shown that low concentrations of the phytohormone stimulate the growth of microalgae, apparently, by reducing the lag phase of their growth. GAhas been found to exert a stimulating effect on the accumulation of carbohydrates, proteins, and chlorophyll. The twofold increase in the density ofthe*T. suecica* culture exposed toGA at a concentration of0.39 $\times$ 10^{--8}M can serve a basis todevelop a technology for culturingmicroalgaeto be used as feed.

References

[1] Kabanova YG. On the cultivation of marine plankton diatoms and peridiniales algae in laboratory conditions. Proceedings of the Institute of Oceanology of the Academy of Sciences of the USSR.. 1961;47:203–216.

[2] Romanenko EA, Kosakovskaya IV, RomanenkoPA. Phytohormones of microalgae: Biological role and involvement in the regulation of physiological processes. Pt I. Auxins, abscisic acid, ethylene.Algologia.2015;25(3):330–351.

[3] Romanenko EA, Kosakovskaya IV, Romanenko PA.Phytohormones of microalgae: Biological role and involvement in the regulation of physiological processes. Pt II. Cytokinins and gibberellins.Algologia.2016;26(2):203–229.

[4] TrenkenshuRP. Calculation of the specific growth rate of microalgae.Marine Biolgy Journale 2019;4(1):100–108.

[5] Aminot A, Ray F. ICES techniques in marine environmental sciences (Iss 30). International Council for the Exploration of the Sea; 2001.Standard procedure for the determination of chlorophyll a by spectroscopic methods. Denmark, Copenhagen, Francisco ReyInstitute of Marine Research

[6] Bentley-Mowat JA, Reid SM. Effect of gibberellins, kinetin and other factors on the growth of unicellular marine algae in culture.Botanica Marina. 1969;12:185—199.

[7] Camacho-Rodríguez J, González-Céspedes AM, Cerón-García MC, Fernández-Sevilla JM, Acién-Fernández FG, Molina-Grima EA. A quantitative study of eicosapentaenoic acid (EPA) production by *Nannochloropsisgaditana* for aquaculture as a function of dilution rate, temperature and average irradiance.Applied Microbiolgy Biotechnolgy 2014;98:2429–2440.

[8] ?arneiro M, Pojo V, Malcata FX, Otero A. Lipid accumulation in selected *Tetraselmis* strains. Journal of Applied Phycology. 2019;31:2845–2853.

[9] Chauton MS, Reitan KI, Norsker NH, Tveterås R, Kleivdal HTA. A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: Research challenges and possibilities. Aquaculture.2015;436:95–103.

[10] Conceição LEC, Yúfera M, Makridis P, Morais S, Dinis MT. Live feeds for early stages of fish rearing. Aquaculture Research. 2010;41:613–640.

[11] Christie W.W. Lipid analysis: Isolation, identification and structural analysis of lipids.Bridgwater: The Oily Press; 2003.

[12] Evans WK, Sorokin C. Studies of the effect of gibberellic acid on algal growth.Life Science 1971;10:1227—1235.

[13] Johnston R. Effects of gibberellin on marine algae in mixed cultures. Limnology and Oceanography.1963;8(2): 270—275.

[14] Herbert D, Phipps PJ, Strange RE. Chemical analysis of microbial cells. Journal of Microbiological Methods1971;58:209-344.

[15] Laurens LML, Dempster TA, Jones HDT et al. Algal biomass constituent analysis: Method uncertainties and investigation of the underlying measuring chemistries. Analytical Chemistry. 2012;84(4):1879–1887.

[16] Lowry O, Rosenbrougt N, Parr A, Randall R. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry. 1951;193(1):265–276.

[17] Mowat JA. A survey of results on the occurrence of auxins and gibberellins in algae.Botanica Marina 1965;8(1):149—155.

[18] Pan X, Chang F, Kang L, Liu Y, Li G, Li D. Effects of gibberellin A3 on growth and microcystin production in *Microcystis aeruginosa* (Cyanophyta). Journal of Plant Physiology.2008;165:1691—1697.

[19] Park W, Yoo G, Moon M, Kim CW, Choi Y, Yang J. Phytohormone supplementation significantly increases growth of *Chlamydomonas reinhardtii* cultivated for biodiesel production.Applied Biochemistry and Biotechnology 2013;171:1128—1142.

[20] Stirk WA, Bálint P, Tarkowská D, Novák O, Strnad M, Ördög V, van Staden J. Hormone profiles in microalgae: Gibberellins and brassinosteroids. Plant Physiology and Biochemistry. 2013;70:348—353.