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ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 1, pp. 56–63
https://marine-biology.ru

UDC 593.161.42-113.4:547.587.11

GROWTH OF *ISOCHRYSIS GALBANA* PARKE, 1949 (HAPTOPHYTA) UNDER MIXOTROPHIC CONDITIONS USING SALICYLIC ACID

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Received by the Editor 28.10.2021; after reviewing 27.04.2022;
accepted for publication 20.10.2022; published online 14.03.2023.

The effect of salicylic acid at different concentrations on growth dynamics of *Isochrysis galbana* Parke, 1949 in the batch culture was estimated. The cultivation was carried out in monoculture. The rise in algal biomass was evaluated by an increase in cell abundance (cells were counted in each experiment in the Goryaev chamber in triplicate under a light microscope). The experiments lasted for 7 days. As found, salicylic acid at concentrations from 2.8×10^{-7} to 5.6×10^{-7} mol·L^{-1} had a stimulating effect on the growth dynamics of *I. galbana* cells, compared with the control group. The maximum cell growth in culture was recorded with the addition of 2.8×10^{-7} mol·L^{-1} of salicylic acid, and the specific growth rate at a given concentration on the 7th day of the experiment was 1.2 times higher than in the control group. Biochemical parameters of *I. galbana* culture with salicylic acid added (2.8×10^{-7} mol·L^{-1}) during 7 days of the experiment were estimated in comparison with parameters of the control group. In the experimental group, the maximum protein content was noted on the 7th day of the experiment. A rise was 76.9% compared to the initial value. As shown, the maximum increase in the content of lipids and carbohydrates in the experimental group occurred on the 5th day. A rise in the values of these indicators was 41.7 and 87%, respectively. Chlorophyll content increased throughout the entire experiment both in the control and experimental groups, and the highest value was registered for the experimental group.

Keywords: microalgae, cultivation, *Isochrysis galbana*, phytohormones, salicylic acid

Marine microalgae play a fundamental role in fish and mollusc feeding, especially in the coastal zones. In aquaculture, one of the most widely used microalgae is *Isochrysis galbana* Parke, 1949 (Haptophyta) – due to its high content of polyunsaturated fatty acids [Sánchez et al., 2013].

I. galbana is mainly used to feed bivalve larvae and early juveniles. For most larvae, the artificial breeding occurs during periods of high temperature; this can alter the lipid composition of *I. galbana* and affect its nutritional value.

Obtaining cultures with a stable composition of nutrients is difficult because of its wide variability under the effect of both techniques and conditions of the media used. Specifically, it was noted as follows: a rise in salinity from 5 to 50 g·L^{-1} reduces the production of total lipids in *I. galbana* by 2 times [Cañavate et al., 2020]. At the same time, an increase in the time of the light regime during cultivation contributes to a rise in docosahexaenoic acid production by the alga by 1.6 times [Tzovenis et al., 1997].

However, cultivation in open water basins is unstable and requires optimization of the main parameters (pH and volume of culture, gas exchange, and flow rate), and this ultimately affects the productivity of photosynthesis [Van Bergeijk et al., 2007]. One of the ways to regulate the cultivation efficiency of microalgae and their biochemical composition is the use of phytohormones. These substances are considered as exogenous bioregulators affecting both the resistance of microalgae to environmental factors and the processes of lipid and pigment biosynthesis [Priyadarshani, Rath, 2012; Romanenko et al., 2016]. However, the effect of phytohormones on different microalgal species may differ greatly. Information on the effect of various groups of these chemical compounds on physiological and biochemical parameters of microalgae remains fragmentary; moreover, the values depend on their concentrations in different growth phases.

Investigations of microalgal phytohormones – food objects for molluscs and invertebrates – are rare and mainly concern the development of methods for their cultivation in order to extract biologically active metabolites (carotenoids and chlorophylls). The issues of the effect of exogenous growth promoters on the cultures of microalgae and their biochemical composition remain understudied. At the same time, knowledge on the physiological effects of phytohormones opens up an industrial perspective for their use on mariculture farms [Kovalev et al., 2021].

The aim of this work was to evaluate the effect of various concentrations of salicylic acid on *I. galbana* growth and biochemical composition in the batch culture.

MATERIAL AND METHODS

We used *I. galbana* culture from the collection of the Research and Production Department of Mariculture of the Far Eastern State Technical Fisheries University. The alga was grown in a batch mode on the nutrient medium f/2. This medium is prepared on the basis of filtered and sterilized seawater, with the addition of solutions of mineral salts ($NaNO_3$; $NaH_2PO_4{\cdot}H_2O$; $Na_2SiO_3{\cdot}9H_2O$), trace elements ($CuSO_4{\cdot}5H_2O$; $ZnSO_4{\cdot}7H_2O$; $CoCl_2{\cdot}6H_2O$; $MnCl_2{\cdot}4H_2O$; $Na_2MoO_4{\cdot}2H_2O$; EDTA-Na_2; $FeCl_3{\cdot}6H_2O$), and vitamins (B_1; B_7; B_{12}) [Guillard, 1975]. The algal culture was maintained under constant conditions: temperature of +21…+23 °C, irradiance of 8–10 klx, photoperiod 8 : 16 h (light : dark), and periodic stirring (4–5 times a day).

In the experiments, salicylic acid (“NevaReaktiv”, Russia) was used as a phytohormone at four concentrations: 2.8×10^{-7}; 5.6×10^{-7}; 8×10^{-7}; and 11.2×10^{-7} mol·L^{-1}. During the experiments, *I. galbana* was kept in 1-L glass heatproof conical flasks. Into sterile flasks, 400 mL of clean filtered and sterilized seawater, 2 mL of the nutrient medium, and 100 mL of algal culture were poured. In four flasks, a phytohormone was added at the beginning of the experiment. The fifth flask was a control: the alga was cultivated with no growth promoters.

The cultivation was carried out in monoculture. A rise in algal biomass was determined by an increase in cell abundance (cells were counted in each experiment in the Goryaev chamber in triplicate under a light microscope). The experiment lasted for 7 days.

To determine the content of total carbohydrates, a sample of algal suspension was subjected to acid hydrolysis. The core of this process is as follows: formed monosaccharide units are converted into furfural derivatives, which form colored compounds upon addition of L-tryptophan to the solution, and those absorb light at a wavelength of 540 nm [Laurens et al., 2012].

Sample preparation for protein determination was carried out according to [Herbert et al., 1971]. Protein content was detected by the Lowry method [Lowry et al., 1951].

Total content of lipids was determined by the method based on the color reaction of vanillin in acidic medium with lipids, with the formation of intense staining. Chromogenic groups are hydroxyl and carbonyl ones [Johnson et al., 1977].

Total chlorophyll was isolated by acetone extraction from pre-frozen algal biomass [Carneiro et al., 2019]. Quantitative chlorophyll content was determined spectrophotometrically at wavelengths of 630, 647, 664, and 750 nm. As a control, 90% acetone was used [Aminot, Ray, 2000].

The specific growth rate was calculated according to [Trenkenshu, Lelekov, 2017].

RESULTS

The effect of different concentrations of salicylic acid on *I. galbana* growth dynamics in the batch culture was analyzed.

As shown, salicylic acid at concentrations from 2.8×10^{-7} to 5.6×10^{-7} mol·L^{-1} stimulated the culture growth. This effect was the greatest at 2.8×10^{-7} mol·L^{-1}: the culture growth was 935.4%. The growth of the control culture during the same period was 744.7% (Fig. 1). Interestingly, the difference between the culture density for *I. galbana* of the control group and the experimental one, grown at 2.8×10^{-7} mol·L^{-1} salicylic acid, was 190.7%, or 1.84 million cells·mL^{-1}.

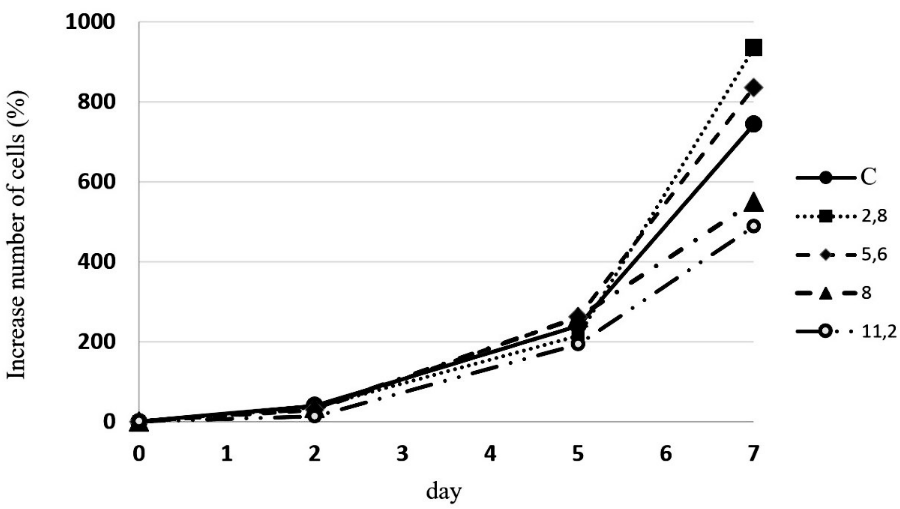

Fig. 1. Growth dynamics of *Isochrysis galbana* culture using salicylic acid (× 10^{-7} mol·L^{-1}) (C denotes control)

Positive values of the specific growth rate indirectly confirm the synthesis rate of the main biochemical components of microalgae. During the initial stages of cell culture growth, there is a shift in the biochemical composition of microalgae; in the exponential phase, the biochemical composition does not change [Trenkenshu, Lelekov, 2017].

The calculation of the specific growth rate (μ) of the microalga showed its linearity in the first 2 days of cultivation. The specific growth rate of the culture during 5 days of exposure to salicylic acid at a concentration of 2.8×10^{-7} mol·L^{-1} did not differ from that for the control group (Fig. 2).

In the experimental group of *I. galbana* cultivated using salicylic acid at 2.8×10^{-7} mol·L^{-1}, on the 7th day, the specific growth rate was 1.2 times higher compared to that for the control group (Fig. 2).

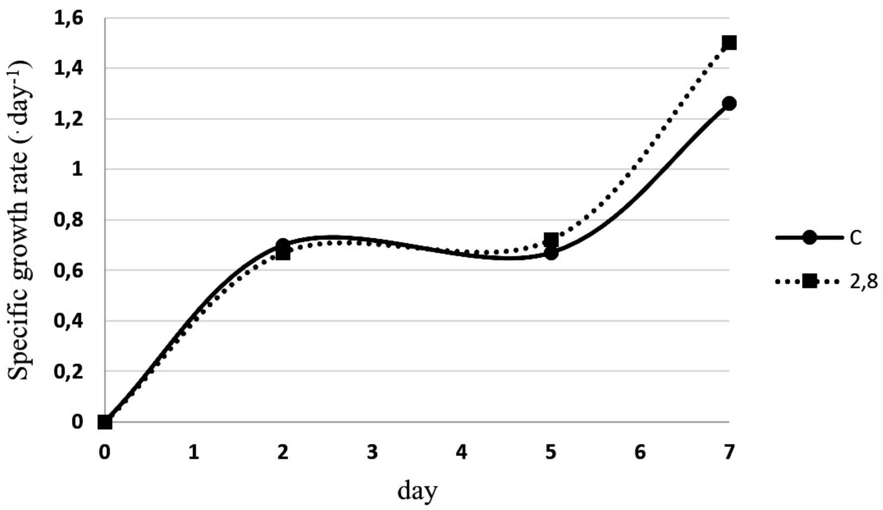

Fig. 2. Specific growth rate of *Isochrysis galbana* culture using salicylic acid (2.8×10^{-7} mol·L^{-1}) (C denotes control)

In the first 5 days of cultivation, in the control group, there was an increase in the protein content by 23%. In the experimental group (with salicylic acid added), a rise in the protein concentration was 69%. Further cultivation of the microalga (up to 7 days) led to a decrease in the protein content in the control group and to an increase by 4.5% in the experimental group (Table 1).

Table 1. Effect of salicylic acid (2.8×10^{-7} mol·L^{-1}) on *Isochrysis galbana* biochemical parameters

Parameter	Protein, µg·mL^{-1}	Carbohydrates, µg·mL^{-1}	Lipids, µg·mL^{-1}	Chlorophyll, µg·mL^{-1}	Culture density, million cells·mL^{-1}
0 days					
C	3.9	10.7	4.8	0.12	0.78
S					
5 days					
C	4.8	13.0	3.9	0.49	1.64
S	6.6	20.1	6.8	0.59	2.02
7 days					
C	4.3	11.5	4.2	0.65	1.89
S	6.9	15.5	6.1	0.76	3.73

Note: C denotes control; S denotes salicylic acid.

The dynamics of changes in the concentration of carbohydrates in culture for 5 days is similar to the dynamics of changes in the protein content. In the control group, an increase by 21.5% was noted; in the experimental group, a rise by 87.9% was recorded. Further cultivation resulted in a decrease in the concentration of carbohydrates in the control group by 11.5%; in the experimental group, there was a drop by 22.9% (Table 1).

The study showed as follows: while cultivating the control group, the content of lipids dropped; the maximum decrease was registered on the 5th day and amounted to 18.8% compared to the concentration of lipids in the initial culture. At the same time, in the culture of *I. galbana* grown with salicylic acid added, an increase in the content of lipids for 5 days was 41.7%. It should be noted that further cultivation was accompanied by a decrease in the value by 10.3% (Table 1).

Positive growth dynamics of chlorophyll content was recorded in both groups during the entire period of cultivation. The increase in chlorophyll concentration in the control group on the 5th day was 308.3%; on the 7th day, it was 441.7%. The values for the experimental group were 391.7 and 533.3%, respectively (Table 1).

DISCUSSION

I. galbana is widely used in aquaculture as a live food. The biochemical composition of microalgae may vary depending on the conditions of their cultivation (growth phase, cultivation mode, temperature, irradiance, nutrient medium composition, *etc.*). It is important to know the biochemical composition of microalgae as a live food. The growth rate and survival of bivalve larvae are known to depend on the quality of the food, and this is determined by the composition of algae – content of protein, carbohydrates, and lipids [Shields, Lupatsch, 2012].

In terms of size and biochemical parameters, the analyzed species is optimal as a part of bivalve diet. However, *I. galbana* cultivation in photobioreactor systems depends on many factors; moreover, it is expensive and unstable in terms of the quantity and quality of biomass produced [Alkhamis, Qin, 2013; Tabelskaya, Kalinina, 2021].

One of the ways to reduce production costs in marine microalgae aquaculture is the use of media supplemented with growth promoters. The use of such regulators is a new strategy for the commercial cultivation of microalgae aimed at improving growth rates and bioproduct synthesis.

Our study showed that salicylic acid stimulated the quantitative growth of *I. galbana* culture by 378%. At the same time, as established earlier, salicylic acid at a concentration of 3.75×10^{-5} mol·L^{-1} stimulated the quantitative growth of the culture of *Tetraselmis suecica* (Kylin) Butcher, 1959 (Chlorophyta) by 415%. Obviously, the physiological effects of salicylic acid on microalgae are species-specific.

During the experiments, the maximum increase in the concentration of carbohydrates and lipids was revealed on the 5th day of cultivation with the use of salicylic acid. At the same time, in the course of previous studies on *I. galbana*, it was found that the use of a medium with the addition of agricultural fertilizers and the nutrient medium f/2 led to a decrease in the content of carbohydrates and lipids during the first 5 days and to accumulation of the maximum amount of protein [Valenzuela-Espinoza et al., 2002]. Our investigations showed that further cultivation (up to 7 days) does not affect the protein concentration in the culture. Interestingly, the growth-promoting concentration of salicylic acid reduced the content of protein and lipids in *T. suecica* with a cultivation period of 14 days.

Lipids are the main source of energy during bivalve larvae development. To complete the metamorphosis of *Magallana gigas* (Thunberg, 1793), the content of lipids in the feed must reach a certain level. When oyster spat feeds on microalgae with high concentration of lipids, a higher growth rate and survival of larvae are recorded. It should be noted as follows: during *I. galbana* cultivation under mixotrophic conditions, the growth and quantitative content of lipids were stimulated by the introduction of glycerol into the medium as an additional carbon source [Danesh et al., 2019].

Chlorophyll content increased during the entire period of *I. galbana* cultivation both in the experimental and control groups. However, a more significant rise in chlorophyll concentration during *I. galbana* cultivation with the addition of salicylic acid indicates an intensifying effect of this stimulant. This, in its turn, affects the biochemical composition of microalgal biomass.

Salicylic acid derivatives stimulate biochemical processes in microalgae as well. Specifically, Madani *et al.* [2020] showed that 2,4-dichloroacetic acid at a concentration of 2 mg·L^{-1} noticeably increased the content of protein and polyunsaturated fatty acids in *I. galbana*.

The data obtained by the same researchers [Madani et al., 2021] indicate the effectiveness of improving *I. galbana* growth when using gibberellic acid at a concentration of 4 mg·L^{-1}. It is noted that an increase in phytohormone concentration from 2 to 6 mg·L^{-1} led to a rise in the concentration of lipids, but to a drop in the content of carbohydrates in terms of the dry weight of the alga. Chlorophyll concentration did not change.

Our study showed that the maximum content of protein, lipids, and carbohydrates was recorded on the 5th day of *I. galbana* cultivation with the addition of 2.8×10^{-7} mol·L^{-1} salicylic acid to the medium. The results obtained can be of practical importance in their industrial application – to optimize the microalga cultivation for mariculture purposes.

Conclusions:

1. The effect of various concentrations of salicylic acid on the growth and biochemical parameters of the mixotrophic culture of *Isochrysis galbana* was evaluated. Salicylic acid at concentrations from 2.8×10^{-7} to 5.6×10^{-7} mol·L^{-1} stimulated the quantitative growth of the microalga cells; at concentrations exceeding 8.0×10^{-7} mol·L^{-1}, it inhibited the growth.
2. The effective concentration of salicylic acid – 2.8×10^{-7} mol·L^{-1} – stimulated the accumulation of protein in the culture by 23% on the 5th day; carbohydrates, by 87.9%; and lipids, by 41.7%.
3. Cultivation of *Isochrysis galbana* culture for more than 5 days was accompanied by a decrease in the parameters of biochemical composition. This must be taken into account when the microalga is used as the feed during the cultivation of invertebrates.

This work was carried out within the framework of the state research assignment No. 121031300015-5.

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РОСТ *ISOCHRYSIS GALBANA* PARKE, 1949 (HAPTOPHYTA) В МИКСОТРОФНЫХ УСЛОВИЯХ С ИСПОЛЬЗОВАНИЕМ САЛИЦИЛОВОЙ КИСЛОТЫ

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Проведена оценка влияния различных концентраций салициловой кислоты на динамику роста *Isochrysis galbana* Parke, 1949 в накопительной культуре. Культивирование осуществляли в монокультуре. Прирост биомассы водорослей находили по увеличению числа клеток, просчитанных в каждом опыте в камере Горяева в трёх повторностях под световым микроскопом. Продолжительность экспериментов составляла 7 суток. Установлено, что концентрации салициловой кислоты от $2{,}8 \times 10^{-7}$ до $5{,}6 \times 10^{-7}$ моль·л$^{-1}$ оказывали стимулирующее воздействие на динамику роста клеток *I. galbana* по сравнению с контрольной группой. Максимальный прирост клеток в культуре отмечен при добавлении салициловой кислоты в концентрации $2{,}8 \times 10^{-7}$ моль·л$^{-1}$, причём удельная скорость роста при данной концентрации на 7-е сутки эксперимента была в 1,2 раза выше, чем в контрольной группе. Проведена оценка биохимических показателей культуры водорослей *I. galbana* с добавлением салициловой кислоты в концентрации $2{,}8 \times 10^{-7}$ моль·л$^{-1}$ в течение 7 суток эксперимента в сравнении с показателями контрольной группы. Максимальное содержание белка в экспериментальной группе зарегистрировано на 7-е сутки опыта. Увеличение составляло 76,9 % по сравнению с начальным значением. Показано, что максимальный рост содержания липидов и углеводов в экспериментальной группе приходился на 5-е сутки опыта. Прирост значений по этим показателям составлял 41,7 и 87 % соответственно. Содержание хлорофилла росло на протяжении всего времени опыта как в контрольной, так и в экспериментальной группе, при этом наибольшее значение показателя отмечено для экспериментальной группы.

Ключевые слова: микроводоросли, культивирование, *Isochrysis galbana*, фитогормоны, салициловая кислота