Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 14, Issue 5, 2024, 106
https://doi.org/10.33263/BRIAC145.106

Cyanobacteria and Microalgae, and the Phenomenon of "Trained Immunity" in Mouse C57Bl/6 Macrophages

Alexander Lykov [1,3*], Ruslan Gevorgiz [2], Svetlana Zheleznova [2], Olga Poveshchenko [1]

1 Research Institute of Clinical and Experimental Lymphology - Branch of the Institute of Cytology and Genetics Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
2 A.O. Kovalevsky Institute of Biology of the Southern Seas of the Russian Academy of Sciences, Sevastopol, Russia
3 Novosibirsk Research Institute of Tuberculosis, Ministry of Health of Russia, Novosibirsk, Russia
* Correspondence: aplykov2@mail.ru

Scopus Athor ID 7005078523

Received: 2.02.2024; Accepted: 12.05.2024; Published: 21.07.2024

Abstract: The effect of extracts of cyanobacteria and microalgae from various taxa and habitats on the establishment of immunity" phenomena in macrophages of C57Bl/6 mice was studied. Peritoneal macrophages were stimulated with an intraperitoneal dose of 1 ml/mouse 4% starch solution or 0.5 mg/mouse BCG vaccination. The metabolic shift in macrophages (glycolysis, lactate production) and the generation of NO and reactive oxygen species were used to determine the "trained immunity" phenomena. In mouse macrophages, glycolysis, lactate synthesis, NO, and the creation of reactive oxygen species were affected by cell pre-priming, cyanobacteria, and microalgae type. The combination of extracts and lipopolysaccharide increased NO production by macrophages in unprimed cells, but priming with BCG vaccination resulted in increased glycolysis and suppression of lactate synthesis, and the opposite pattern was observed when macrophages were primed with starch. As a result, we found no evidence of a typical metabolic rearrangement in peritoneal macrophages from C57Bl/6 mice.

Keywords: cyanobacteria; microalgae; macrophage; glycolysis; lactate; nitric oxide; reactive oxygen species.

1. Introduction

After initial encounters with pathogens, BCG vaccination, or β-glucan, macrophages (Mf) develop "trained immunity" (TI) through epigenetic and metabolic changes [1-4]. Through interaction with TLR2 and TLR4, Algal polysaccharides can also cause TI in Mf. Thus, in vitro, algal extracts exert a short-term anti-inflammatory effect in pig peripheral blood monocytes and alveolar Mf while inhibiting viral multiplication [5]. The ability of cyanobacteria and microalgae to create the phenomena of TI has not been studied. The study sought to evaluate the effect of cyanobacteria and microalgae extracts on the generation of TI in male C57Bl/6 Mf *in vitro*.

2. Materials and Methods

2.1. Plant material.

The cyanobacteria and microalgae from a variety of systematic groups that were collected from the A.O. Kovalevsky Institute of Biology of the Southern Seas of the RAS and cyanobacteria from the cave (Roholtiella mixta sp. nov.) were used in this experiments for

induction of TI in mouse Mf. The marine cyanobacteria *Leptolyngbya cf. ectocarpi* (*L. ectocarpi*) and freshwater cyanobacteria *Planktothrix agardhii* (*P. agardhii*) and *Arthrospira* (Spirulina) *platensis* (*A. platensis*), and soil cyanobacteria *Roholtiella* mixta sp. nov. (*R. mixta sp. nov.*), diatoms *Nanofrustulum shiloi* (*N. shiloi*) and green microalgae *Tetraselmis* (*Platymonas*) *viridis* Rouchijanen (*T. viridis*) were include in study.

2.2. Preparation of cyanobacteria and microalgae extracts.

The 1 g of dried biomass of cyanobacteria and microalgae was mixed with 1% dimethyl sulfoxide solution (DMSO), and bioactive molecules were extracted by passive diffusion for 24 hours at 37°C. Next, the suspension of cyanobacteria and microalgae in 1% DMSO solution was precipitated for 10 minutes at 3000 rpm and filtered through millipore syringe tips (Merck, USA).

2.3. Induction of trained immunity in mice.

Studies with laboratory animals were carried out in compliance with humane treatment standards and with the consent of the local ethical committee of Novosibirsk Tuberculosis Research Institute of the Ministry of Health of Russia (protocol No. 56 of May 27, 2023).

Resident peritoneal Mf (pMf) "primed" with 1 mL/mouse of 4% starch solution or 0.5 mg/mouse of BCG vaccination strain (Microgen, Russia) intraperitoneally were collected from abdominal cavity lavage fluid. 10^6 pMf/well in RPMI 1640 nutrient medium (Biolot, Russia) supplemented with 10% FCS (Hyclone, USA), 2 mM L-glutamine (Merck, USA), 5 mM HEPES buffer (Sigma, USA), and 1% antibiotic/antimycotic (Invitrogen, USA) were added to 24-well flat-bottom plates (TPP, Switzerland) for 24 hours at 37°C and 5% CO_2. Non-adherent cells were removed, and a fresh medium was added for 72 hours. The medium was changed, and a 1% extract of cyanobacteria and microalgae without and with 100 ng/mL LPS (Sigma, USA) was added to a part of the wells at 100 µL each.

After 24 hours, the conditioned medium was collected to analyze glucose consumption and lactate generation using commercial kits Lactate-Novo and Glucose-Novo (Vector-Best, Russia), as well as persistent nitric oxide (NO) metabolites using Griess reagent on a spectrophotometer.

After adding 100 µL of 0.2% nitro blue tetrazole solution (NST, Sigma, USA) to the remaining pMf in the wells for 30 minutes, the reagent was removed, and the wells were washed. Next, 100 µL of dimethyl sulfoxide/10% KOH mixture (Sigma, USA) was added, and the intensity of the reaction was evaluated spectrophotometrically (INNO-S, LTek, South Korea).

2.4. Statistical analysis.

The data was statistically processed using the Statistica 10 application. The data was evaluated for normal distribution using the Shapiro-Wilks w-criterion. Mean and standard deviation (M±SD) were used in the tables. One-factor analysis of variance (ANOVA) with Bonferroni correction was used to assess statistical significance between samples. P-values < 0.05 were accepted.

3. Results and Discussion

3.1. The effect of cyanobacteria and microalgae DMSO extracts on resident peritoneal macrophages activity.

Previously, we demonstrated the existence of carbohydrates in the composition of cyanobacteria and microalgae, with concentrations ranging from 50 to 340 mg/g dry biomass [6]. Increased glycolysis and lactate production are some of the indications of TI in Mf [2].

Including 1% cyanobacteria and microalgae extracts in the nutritional medium had varying impacts on the amount of glucose absorbed by resident Mf. Thus, extracts from cyanobacteria *L. carpii* and *R. mixta* considerably reduced glycolysis, whereas other cyanobacteria *T. agardhii* and *A. platensis*, as well as microalgae *T. viridis* and *N. shiloi*, boosted glycolysis by Mf (Table 1).

Table 1. Effect of DMSO extracts from cyanobacteria and microalga on resident peritoneal macrophage.

Parameters	Control	Cyanobacteria				Microalgae	
		L. ectocarpi	*R. mixta*	*T. agardhii*	*A. platensis*	*T. viridis*	*N. shiloi*
Glycolisis (mM)							
Basal	5.36±0.02	5.63±0.05*	5.16±0.06*	8.77±0.15*	4.97±0.03*	4.98±0.04*	4.75±0.05*
LPS	5.31±0.04	4.1±0.04*#	4.82±0.01*#	8.82±0.14*	5.62±0.04*#	4.85±0.03*	5.39±0.04#
Lactate (mM)							
Basal	2.39±0.01	5.09±0.02*	1.55±0.01*	2.52±0.02*	2.54±0.01*	4.59±0.03 *	2.51±0.03*
LPS	6.2±0.9#	5.23±0.02#*	1.64±0.01*#	2.17±0.02*#	2.22±0.01*#	3.51±0.01*#	1.86±0.01*#
NO (micromole/mL)							
Basal	4.47±0.05	17.38±0.24*	5.67±0.05*	13.03±1.95*	17.77±0.28*	5.54±0.09*	5.42±0.14*
LPS	4.65±0.05#	18.6±0.3*#	5.48±0.05*#	13.64±0.54*	20.51±0.32*#	5.73±0.42*	5.14±0.05*#
Generation of reactive oxygen species (OD at 630 nm)							
Basal	0.1±0.001	0.1±0.001	0.1±0.001	0.12±0.001*	0.1±0.001	0.15±0.001*	0.11±0.001
LPS	0.11±0.001#	0.12±0.001*#	0.1±0.001	0.1±0.001#	0.1±0.001	0.15±0.001*	0.1±0.001#

Data are expressed as mean, standard deviation (n=3), and significant differences at $P < 0.05$ *with basal or LPS-stimulated level; #with LPS-stimulated level. LPS, lipopolysaccharide.

At the same time, when macrophages are stimulated by LPS, extracts of most cyanobacteria and microalgae, except for cyanobacteria *T. agardhii* and *A. platensis*, dramatically accelerate glycolysis.

Increased lactate generation by innate immune cells is another important hallmark of metabolic rearrangement in TI [1, 2]. Resident mouse pMf produces considerably more lactate in response to LPS stimulation. Non-primed pMf produce more lactate in response to cyanobacteria and microalgae extracts, with the exception of the cyanobacterium *R. mixta*. However, in the presence of LPS, lactate generation by resident pMf is considerably decreased.

Nitric oxide, an antibacterial chemical produced by innate immune cells, is also higher in cells with the TI phenotype [1]. In control macrophages, LPS stimulation does not considerably boost NO synthesis. In contrast, cyanobacteria and microalgae extracts in the nutritional medium, both in the absence and presence of LPS, greatly stimulate NO production.

It was discovered that macrophages with the TI phenotype produce more reactive oxygen species (ROS), which are important for cell antibacterial activity [1]. LPS has been found to increase the production of ROS by resident mice peritoneal macrophages, except for *T. viridis*, cyanobacteria, and microalgae extracts had no significant effect on the production of ROS.

3.2. The impact of extracts from cyanobacteria and microalgae on BCG-primed macrophages.

The creation of long-term non-specific immunological memory for the BCG vaccine in myeloid cells is well documented; however, it has not survived for more than a year. On the other hand, it has been discovered that BCG-vaccinated individuals, particularly newborns, may withstand viral infections better and die from them less frequently. This encourages the hunt for active biological compounds that can enhance the effect of the BCG vaccine on TI development [1].

Table 2. Impact of DMSO extracts from cyanobacteria and microalgae on peritoneal macrophages with Trained immunity induced by BCG vaccine.

Parameters	**Control**	**Cyanobacteria**				**Microalgae**	
		L. ectocarpi	***R. mixta***	***T. agardhii***	***A. platensis***	***T. viridis***	***N. shiloi***
Glycolisis (mM)							
Basal	5.65±0.3	5.31±0.02*	5.72±0.03*	6.15±0.02*	5.51±0.01*	4.46±0.04*	5.06±0.03*
LPS	5.11±0.01#	5.4±0.05*#	5.42±0.21*#	5.73±0.05*#	5.56±0.01*#	4.71±0.02*#	5.31±0.02*#
Lactate (mM)							
Basal	3.03±0.01	4.43±0.02*	1.87±0.01*	2.61±0.02*	4.56±.13*	4.93±0.04*	3.35±0.03*
LPS	4.43±0.18#	4.26±0.02#	1.89±0.02*	3.59±0.21*#	2.78±0.29*#	5.37±0.03*#	4.31±0.05#
NO (micromole/mL)							
Basal	5.02±0.37	18.29±0.18*	5.79±0.11*	12.78±0.19*	18.54±0.59*	7.76±0.33*	5.94±0.05*
LPS	5.82±0.18#	21.11±0.14*#	5.91±0.16	14.94±0.23*#	20.27±0.9*#	7.52±0.32*	5.48±0.05*#
Generation of reactive oxygen species (OD at 630 nm)							
Basal	0.1±0.001	0.11±0.001*	0.08±0.001*	0.08±0.001*	0.12±0.001*	0.12±0.001*	0.09±0.00*
LPS	0.12±0.001#	0.12±0.001#	0.09±0.001*#	0.1±0.01*#	0.12±0.001	0.11±0.001*#	0.11±0.001*#

Data are expressed as mean, standard deviation (n=3), and significant differences at $P < 0.05$ *with basal or LPS-stimulated level; #with LPS-stimulated level. LPS, lipopolysaccharide.

To that goal, we primed mice peritoneal macrophages with BCG vaccination and assessed the influence of extracts from tested cyanobacteria and microalgae on key indicators of TI cell phenotype development (Table 2).

In contrast, in controls, BCG vaccine-primed macrophages markedly reduced glycolysis while increasing lactate synthesis, NO production, and the creation of ROS in response to LPS stimulation.

Only the cyanobacteria *R. mixta* and *A. platensis* inhibited glycolysis of macrophages in the absence of LPS, but other cyanobacteria and microalgae, on the contrary, boosted glucose absorption by macrophages. Except for *T. viridis*, most cyanobacteria and microalgae did not increase macrophage glycolysis in the presence of LPS.

At the same time, cyanobacteria and microalgae, with the exception of *R. mixta* and *T. agardhii*, dramatically boosted lactate generation by macrophages in the absence of LPS, whereas the presence of LPS in the culture medium reduced lactate production.

We observed an increase in NO production by macrophages in the absence and presence of LPS in the nutritional medium in response to cyanobacteria and microalga extracts. In addition, cyanobacteria and microalgae extracts had no significant effect on the formation of ROS.

3.3. The effect of cyanobacteria and microalgae extract on starch hydrolysate primed macrophages.

Cyanobacteria and microalgae extracts increased glycolysis of primed hydrolyzed starch macrophages in the absence and, to a lesser extent, in the presence of LPS in the culture medium (Table 3).

Table 3. Effect of DMSO extracts from cyanobacteria and microalgae on peritoneal macrophages primed with 4% starch solution.

Parameters	**Control**	**Cyanobacteria**			**Microalgae**		
		L. ectocarpi	***R. mixta***	***T. agardhii***	***A. platensis***	***T. viridis***	***N. shiloi***
Glycolisis (mM)							
Basal	5.65±0.05	4.47±0.48*	5.53±0.07	5.09±0.06*	5.08±0.06*	4.67±0.04*	4.82±0.05*
LPS	6.8±0.05#	5.03±0.05*#	5.58±0.06*#	4.98±0.05*	5.08±0.06*	4.28±0.05*#	4.55±0.04*#
Lactate (mM)							
Basal	3.79±0.01	7.31±0.02*	3.85±0.08	5.02±0.02*	5.6±0.01*	6.35±0.02*	5.98±0.02*
LPS	4±0.28	6.64±0.03*#	5.78±0.24*#	5.01±0.19*	5.47±0.0*#	8.19±0.1*#	6.24±0.03*#
NO (micromole/mL)							
Basal	4.68±0.05	18.17±0.3*	5.7±0.05*	12.13±0.14*	17.03±0.59*	5.76±0.05*	5.42±0.14*
LPS	5.88±0.11#	16.51±0.14*#	5.33±0.05*#	5.24±0.14*#	17.46±0.67*	7.58±0.16*	13.03±0.33*#
Generation of reactive oxygen species (OD at 630 nm)							
Basal	0.13±0.001	0.14±0.001*	0.18±0.001*	0.11±0.001*	0.18±0.01*	0.14±0.001*	0.14±0.001*
LPS	0.16±0.001#	0.12±0.001*#	0.18±0.001*	0.12±0.001*#	0.19±0.001*#	0.14±0.001*	0.15±0.001*#

Data are expressed as mean, standard deviation (n=3), and significant differences at $P < 0.05$ *with basal or LPS-stimulated level; #with LPS-stimulated level. LPS, lipopolysaccharide.

Furthermore, extracts of cyanobacteria and microalgae increased the synthesis of lactate, NO, and, in most cases, ROS by starch-primed mouse macrophages in the absence and presence of an LPS stimulus in the culture medium, respectively.

Our study included cyanobacteria and microalgae from various taxonomic groups and environments. We discovered that the degree of glycolysis by unprimed mouse pMf varied according to the type of cyanobacteria and microalgae. At the same time, pMf mice primed with BCG vaccination or starch showed lower glycolysis intensity only in the presence of P. agardhii extract in the medium. The decrease in pMf glycolysis intensity could be attributed to the fact that the total sugar content of the culture medium rose by at least 1 mM in the presence of cyanobacteria and microalgae extracts when compared to the control parameter. This resulted in a decrease in lactate production by unprimed mouse pMf. Still, BCG vaccination primed mouse pMf increased lactate production in response to stimulation with cyanobacteria and microalgae extracts without an LPS stimulus. This could be due to the fact that polysaccharides found in cyanobacteria and microalgae extracts can act as a second stimulus, and the addition of LPS causes resistance to form in pMf, resulting in a decrease in metabolic rearrangement.

In contrast, starch priming of mice pMf increases lactate production in spontaneous and LPS-stimulated testing, most likely due to a lack of competition between polysaccharide and LPS stimuli.

Most cyanobacteria and microalgae increase NO production through pMf. Still, no such effect was observed in ROS synthesis except for *P. viridis* extract, which may indicate the antibacterial potential of such cells. The decrease in ROS generation could be attributed to the presence of vitamins, polysaccharides, and trace elements in cyanobacteria and microalgae extracts, all of which function as antioxidants.

Microalgae produce autotrophs, which are considered a source of a wide range of biologically active compounds such as proteins, lipids, and carbohydrates used in various industrial applications. Unsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid have been detected in the lipid composition of microalgae, and they influence the antibacterial, anti-inflammatory, and immunological responses of humans and animals [7]. Innate immunity is the initial line of defense for humans and animals against infectious diseases. There is evidence of the effect of microalgae on the inflammatory response; however, the mechanisms behind the therapeutic action have not been thoroughly studied. β-glucans boost immune function and resistance to infections [8].

Cyanobacteria and microalgae contain substantial amounts of polysaccharides, which, like lipopolysaccharides, can stimulate an immunological response in immunocytes. Exopolysaccharides produced by cyanobacteria and microalgae have immunostimulatory properties [9]. For example, sulfated polysaccharides from the filamentous microalgae *Tribonema sp*., predominantly galactose, stimulate RAW264.7 macrophages to generate IL-6, IL-10, and TNF-α [10]. Exopolysaccharides from *Dunaliella salina* as ethyl acetate fraction stimulated IFN-γ, TNF-α, and TGF-β production by peripheral blood mononuclear cells and RAW 264.7 macrophages [11]. Some polysaccharides are classified as "biological response modifiers", which are biomolecules that boost the immune response and can come from various sources. Chemical analysis of polysaccharides isolated from the marine diatom alga *Conticribra weissflogii* revealed the presence of (1 → 3)-linked β-D-glucan with a low proportion of C-6 substitution by single β-glucose units, which enhanced the phagocytic potential of macrophages against glioblastoma cell lines (U87 MG and U251) and RAW 264.7 macrophages [12].

We did not chemically analyze the extracts for cyanobacteria or microalgae β-glucans. However, it has been previously proven that cyanobacteria and microalgae have a considerable amount of carbohydrates, which may include β-glucans [6]. At the same time, different carbohydrates can influence the immune system's cell function. Studies conducted by other authors support this. *Tribonema microalgae* can accumulate chrysolaminarin (polysaccharide), which at a concentration of 1 mg/mL induces phagocytosis and mRNA production of IL-1β, IL6, TNF-α, and Nos2, increases phosphorylation of p-65, p-38, and JNK in NF-κB and MAPK signaling pathways [13].

Lipids also affect the functional features of immune system cells. Cyanobacteria and microalgae are excellent sources of long-chain polyunsaturated fats. We previously demonstrated the presence of diverse quantities of polyunsaturated fatty acids in cyanobacteria and microalgae, including eicosapentaenoic acid, arachidonic acid, γ-linolenic acid, linolenic acid, oleic acid, and α-linolenic acid [6]. Digomo-γ-linolenic acid from green microalga *Lobosphaera incisa* triggered the formation of prostaglandin PGE_1 by RAW264.7 cells but did not influence the synthesis of pro-inflammatory cytokines in response to LPS stimulation. It also greatly reduced the generation of ROS and NO [14].

Innate immunity cells respond to stimuli by producing pro-inflammatory cytokines such as L-1β, IL-6, and TNF-α. Cytokines are produced and secreted primarily through the NF-κB and NLRP3 inflammasome signaling pathways. The essence of innate immune cells with a TI phenotype is the fast overproduction of pro-inflammatory cytokines to increase the immune response to a second contact with an initial stimulus or activation by other antigens. In recent years, researchers have focused on the impact of microalgae extracts on the generation of cytokines and other active molecules implicated in inflammation start, as well as their

antibacterial or antitumor properties. We investigated the effect of mice consuming food soaked in oil extracts of *C. vulgaris*, *Coelastrella sp.*, *A. platensis*, *C. closterium*, and *P. purpureum* on the production of NO, IL-1β, IL-10, and TNF-α at the systemic level (serum) as well as in conditioned media from splenocytes and thymocytes. Dietary intake of cyanobacteria and microalgae extracts affected blood IL-1β and TNF-α levels, as well as immune cell production of IL-1β, TNF-α, and IL-10 [15, 16].

Algal extracts decrease NF-κB and NLRP3 activation, increasing intracellular potential and decreasing ROS and lipid peroxidation in macrophages [17, 18]. *Tetraselmis sp.* extract inhibits NO generation in RAW264.7 cells after LPS stimulation by suppressing increased NO synthase expression and releasing TNFα, IL-6, and IL-1β via MAPK and NF-κB-dependent signaling processes [19]. Takahashi *et al.* report that an alcoholic extract of *Botryococcus terribilis* decreased NO, Ccl2, Cox2, and IL-6 production while increasing Pgc1β and Socs1 production in the murine macrophage line RAW264 [20]. This study found that an alcoholic extract of the Antarctic freshwater microalga *Micractinium simplicissimum* regulates COX-2, IL-6, iNOS, TNF-α, and NO synthase activities in the RAW 264.7 macrophage line [21].

The safety of cyanobacteria and microalgae to humans and animals is equally critical. The authors discovered no effect on B-cells and monocytes/macrophages of microalgae. Still, they noted a shift in the relative composition of CD4+ T-cells and CD8- TCR γδ T-cells, indicating the safety of incorporating *Chlorella vulgaris* or *Tetradesmus obliquus* into chicken diets [22]. We also discovered no major harmful effects of consuming oil extracts of several cyanobacteria and microalgae in the diet [16]. Our study's limitations include the inability to isolate purified lipid and sugar fractions and the inability to assess the influence of extracts on cytokine production levels. These points will be investigated more in the future.

4. Conclusions

In this study, we investigated the effect of DMSO extracts of cyanobacteria and microalgae on peritoneal macrophages with the TI phenotype, and the analysis and discussion of the findings led us to the following conclusions: culturing unprimed peritoneal macrophages with cyanobacteria and microalgae extracts partially activates glycolysis but does not increase lactate synthesis. However, they significantly increase nitric oxide synthesis; extracts of cyanobacteria and microalgae on peritoneal macrophages with TI phenotype induced by BCG *in vivo* vaccine mostly do not stimulate glycolysis but increase lactate and nitric oxide production; in the presence of LPS, peritoneal macrophages with TI phenotype respond to cyanobacteria and microalgae extracts, which increase nitric oxide synthesis. This could be due to the competitive effect of the extracts on cells; priming peritoneal macrophages with hydrolyzed starch promotes lactate synthesis, nitric oxide synthesis, and reaction generation.

Funding

This research was performed within the framework of the state assignment registration numbers No. 1023022100045-0, No. 122022800132-1, and No. 121030300149-0.

Acknowledgments

We are grateful to Goncharov A.A. (Director of the Federal Research Center for Biodiversity of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia) for sharing the sample of the Roholtiella mixta sp. nov. cyanobacterium sample.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bekkering, S.; Blok Bastiaan, A.; Joosten Leo, A.B.; Riksen Niels, P.; van Crevel, R.; Netea Mihai, G. *In Vitro* Experimental Model of Trained Innate Immunity in Human Primary Monocytes. *Clin. Immunol. Rev.* **2016**, *23*, 926-933, https://doi.org/10.1128/CVI.00349-16.
2. Lykov, A.P.; Belogorodtsev, S.N.; Nemkova, E.K.; Vetlugina, A.; Terekhova, T.M.; Schwartz, Y.S. The Formation of Non-Specific Immunological Memory Phenotype in Human Monocyte-Like THP-1 and U-937 Cell Lines. *Bull. Exp. Biol. Med*. **2023**, *175*, 477-480, https://doi.org/10.1007/s10517-023-05890-3.
3. De Marco Castro, E.; Calder, P.C.; Roche, H.M. β-1,3/1,6-Glucans and Immunity: State of the Art and Future Directions. *Mol. Nutr. Food Res*. **2021**, *65*, 1901071, https://doi.org/10.1002/mnfr.201901071.
4. Besednova, N.N.; Zaporozhets, T.S.; Kuznetsova, T.A.; Makarenkova, I.D.; Kryzhanovsky, S.P.; Fedyanina, L.N.; Ermakova, S.P. Extracts and Marine Algae Polysaccharides in Therapy and Prevention of Inflammatory Diseases of the Intestine. *Mar. Drugs* **2020**, *18*, 289, https://doi.org/10.3390/md18060289.
5. Hervet, C.; Bussy, F.; Le Goff, C.; Ménard, D.; Collén, P.N.; Goff, M.L.; Meurens, F.; Bertho, N. Marine-Sulfated Polysaccharides Extracts Exhibit Contrasted Time-Dependent Immunomodulatory and Antiviral Properties on Porcine Monocytes and Alveolar Macrophages. *Animals* **2022**, *12*, 2576, https://doi.org/10.3390/ani12192576.
6. Lykov, A.; Salmin, A.; Gevorgiz, R.; Zheleznova, S.; Rachkovskaya, L.; Surovtseva, M.; Poveshchenko, O. Study of the Antimicrobial Potential of the *Arthrospira platensis*, *Planktothrix agardhii*, *Leptolyngbya* cf. *ectocarpi*, *Roholtiella mixta* nov., *Tetraselmis viridis*, and *Nanofrustulum shiloi* against Gram-Positive, Gram-Negative Bacteria, and Mycobacteria. *Mar. Drugs* **2023**, *21*, 492, https://doi.org/10.3390/md21090492.
7. Zhou, J.; Wang, M.; Saraiva, J.A.; Martins, A.P.; Pinto, C.A.; Prieto, M.A.; Simal-Gandara, J.; Cao, H.; Xiao, J.; Barba, F.J. Extraction of lipids from microalgae using classical and innovative approaches. *Food Chem*. **2022**, *384*, 132236, https://doi.org/10.1016/j.foodchem.2022.132236.
8. Teixeira, C.; Peixoto, D.; Hinzmann, M.; Santos, P.; Ferreira, I.; Pereira, G.V.; Dias, J.; Costas, B. Dietary Strategies to Modulate the Health Condition and Immune Responses in Gilthead Seabream (*Sparus aurata*) Juveniles Following Intestinal Inflammation. *Animals* **2022**, *12*, 3019, https://doi.org/10.3390/ani12213019.
9. Risjani, Y.; Mutmainnah, N.; Manurung, P.; Wulan, S.N.; Yunianta. Exopolysaccharide from *Porphyridium cruentum* (*purpureum*) is Not Toxic and Stimulates Immune Response against Vibriosis: The Assessment Using Zebrafish and White Shrimp *Litopenaeus vannamei*. *Mar. Drugs* **2021**, *19*, 133, https://doi.org/10.3390/md19030133.
10. Chen, X.; Song, L.; Wang, H.; Liu, S.; Yu, H.; Wang, X.; Li, R.; Liu, T.; Li, P. Partial Characterization, the Immune Modulation and Anticancer Activities of Sulfated Polysaccharides from Filamentous Microalgae *Tribonema* sp. *Molecules* **2019**, *24*, 322, https://doi.org/10.3390/molecules24020322.
11. Goyal, M.; Baranwal, M.; Pandey, S.K.; Reddy, M.S. Hetero-Polysaccharides Secreted from *Dunaliella salina* Exhibit Immunomodulatory Activity Against Peripheral Blood Mononuclear Cells and RAW 264.7 Macrophages. *Indian J. Microbiol.* **2019**, *59*, 428-435, https://doi.org/10.1007/s12088-019-00818-w.
12. Rizzi, J.; Moro, T.R.; Winnischofer, S.M.B.; Colusse, G.A.; Tamiello, C.S.; Trombetta-Lima, M.; Noleto, G.R.; Dolga, A.M.; Duarte, M.E.R.; Noseda, M.D. Chemical structure and biological activity of the (1 → 3)-linked β-D-glucan isolated from marine diatom *Conticribra weissflogii*. *Int. J. Biol. Macromol.* **2023**, *224*, 584-593, https://doi.org/10.1016/j.ijbiomac.2022.10.147.
13. Wang, F.; Yang, R.; Guo, Y.; Zhang, C. Isolation, Characterization and Immunomodulatory Activity Evaluation of Chrysolaminarin from the Filamentous Microalga *Tribonema aequale*. *Mar. Drugs* **2023**, *21*, 13, https://doi.org/10.3390/md21010013.
14. Novichkova, E.; Chumin, K.; Eretz-Kdosha, N.; Boussiba, S.; Gopas, J.; Cohen, G.; Khozin-Goldberg, I. DGLA from the Microalga *Lobosphaera Incsa* P127 Modulates Inflammatory Response, Inhibits *iNOS* Expression and Alleviates NO Secretion in RAW264.7 Murine Macrophages. *Nutrients* **2020**, *12*, 2892, https://doi.org/10.3390/nu12092892.
15. Lykov, A.P.; Uvarov, I.P.; Gevorgiz, R.G.; Zheleznova, S.N. Effect of microalgae extracts on cytokine levels in female C57Bl6 mice. [Влияние экстрактов микроводорослей на уровни цитокинов у мышей-самок C57Bl6]. *Med. Immunol.* (Russia). **2023**, *25*, 81-90, https://doi.org/10.15789/1563-0625-EOE-2379.

16. Lykov, A.; Uvarov, I.; Gevorgiz, R.; Zheleznova, S.; Surovtseva, M.; Kim, I.; Bondarenko, N.; Poveshchenko, O. Bioavailability and Safety of Lipid Fraction from Different Taxa of Microalgae in Female C57BL/6 Mice. *Biointerface Res. Appl. Chem.* **2022**, *12*, 6845-6862, https://doi.org/10.33263/BRIAC125.68456862.
17. Brun, P.; Piovan, A.; Caniato, R.; Dalla Costa, V.; Pauletto, A.; Filippini, R. Anti-Inflammatory Activities of *Euglena gracilis* Extracts. *Microorganisms* **2021**, *9*, 2058, https://doi.org/10.3390/microorganisms9102058.
18. Lee, A.H.; Shin, H.-Y.; Park, J.-H.; Koo, S.Y.; Kim, S.M.; Yang, S.-H. Fucoxanthin from microalgae Phaeodactylum tricornutum inhibits pro-inflammatory cytokines by regulating both NF-κB and NLRP3 inflammasome activation. *Sci. Rep*. **2021**, *11*, 543, https://doi.org/10.1038/s41598-020-80748-6.
19. Kim, E.-A.; Kang, N.; Heo, S.-Y.; Oh, J.-Y.; Lee, S.-H.; Cha, S.-H.; Kim, W.-K.; Heo, S.-J. Antioxidant, Antiviral, and Anti-Inflammatory Activities of Lutein-Enriched Extract of *Tetraselmis* Species. *Mar. Drug*s **2023**, *21*, 369, https://doi.org/10.3390/md21070369.
20. Takahashi, S.; Ferdousi, F.; Yamamoto, S.; Hirano, A.; Nukaga, S.; Nozaki, H.; Isoda, H. *Botryococcus terribilis* Ethanol Extract Exerts Anti-inflammatory Effects on Murine RAW264 Cells. *Int. J. Mol. Sci*. **2023**, *24*, 6666, https://doi.org/10.3390/ijms24076666.
21. Chae, H.-J.; Seo, J.B.; Kim, S.-H.; Youn, E.J.; Kim, S.; Suh, S.-S. Antarctic Freshwater Microalga, *Micractinium simplicissimum*, Suppresses Inflammation. *J. Nanosci. Nanotechnol*. **2021**, *21*, 4098-4103, https://doi.org/10.1166/jnn.2021.19158.
22. Kim, Y.-B.; Park, J.; Heo, Y.-J.; Lee, H.-G.; Kwon, B.-Y.; Joo, S.S.; Joo, S.Y.; Kim, M.; Kim, Z.-H.; Lee, K.-W. Effect of Dietary *Chlorella vulgaris* or *Tetradesmus obliquus* on Laying Performance and Intestinal Immune Cell Parameters. *Animals* **2023**, *13*, 1589, https://doi.org/10.3390/ani13101589.