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Biologically Active Microalgae and Cyanobacteria in Nature and Marine Biotechnology

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Abstract

There are no less than 100 producers of toxic and biologically active/biocidal substances among 1100 cyanobacterial and microalgal species in the Black Sea. Our investigations include two aspects: (1) evaluation of ecological and biochemical connections between cyanobacteria and microalgae and herbivorous organisms ("grazers"), and (2) formation of new approaches to the using of hydrobionts and their metabolites in human affairs. Experimental data confirm that biological and ecological constructiveness of mass and active cyanobacteria and microalgae species lays not only in toxic effect. It is complex, multiple and many-sided challenge and a part of co-evolutionary process, which resides very close to allelochemical interactions between plants and herbivorous organisms in terrestrial ecosystems. Selectivity of biocidal effect of cyanobacteria and microalgae and high level of their controlled cultivation make possible to create new preparations for pest control.

Keywords: microalgae, cyanobacteria, Black Sea, biocidal activity, biotechnology.

Introduction

Cyanobacteria and microalgae form the first trophic level and the base of interspecific relationship in aqueous ecosystems. There are no less than 100 producers of toxic and biologically active/biocidal substances among 1100 cyanobacteria and microalgae species in the Black Sea and the Sea of Azov. Some of them are known as toxic or HAB objects in different parts of the world, the others are responsible for mass propagation and "red tides" in the coastal Black Sea area or "water blooms" in seashore salt lakes, ponds and estuaries. Their number demonstrates the up-trend during last decades (e.g. dinoflagellates increased this rate from 17 to 53 species since 1948) (Morozova-Vodyanitskaya, 1948; Gomez, Boicenco, 2004; Terenko, 2005) due the anthropogenic factor. These processes present significant environmental impact on water quality, state of marine biota (microorganisms, zooplankton, fishes, marine mammals) and human diseases in global and local dimensions, but they are known insufficiently (firstly inventory and identification of toxins and biocidal substances of mass cyanobacterial and microalgal species, their impact on interspecific ties, ecological pathology, etc.) (Hallegraef, 2003).

It is our understanding based on the last experimental data, that biological and ecological significance of the mass cyanobacteria and microalgae consists not only in toxic effects, but also lays in the sphere of complex and multilateral co-evolution process, therefore terms "toxic" or "potentially toxic" are not quite correct to some mass species, and the concepts "biologically active", "biocidal" or "defensive character" are more preferable in these cases. The level of toxicity or producing of toxins/biologically active substances by non-toxic species or strains may be significantly associated with unfavourable environmental situation, disbalance of ecosystem, intrusion of antagonists, competitors or plant-feeders, etc. Cases of hydrobionts morality can be explained by influence of toxic decomposition products of cyanobacterial/microalgal excessive biomass. We regard cyanobacterial and microalgal secondary metabolites in the light of their defensive action, these substances stand in marked contrast to known biotoxins striking vertebrate animals during "water blooms" and "red tides" (Gol'din, 2009). The defensive activity has a disastrous influence on vital functions of competitors and/or plant-feeders, and causes stress, repellent and deterrent effects, but not obligatory death (Cembella, 2003; Amsler, 2008; Berry *et al*., 2008). Cyanobacterial and microalgal

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responses are very close to inhibitory activity of terrestrial plants-producers of allelochemics for the defense from the other plants or herbivorous arthropods and microbial pathogens (Gol'din, 1995; 2009; Gol'din and Gol'dina, 2002; 2004; Hay *et al*., 1987; Paul *et al*., 2007; Berge *et al*., 2010).

In this light cyanobacterial and microalgal biocidal metabolites may be a natural source of agricultural and medical preparations for the biological control of harmful organisms as one of the most likely ways in this trend. From practical perspective one of the most promising ways of cyanobacteria and microalgae using is the sphere of plant protection and pathogen-pest control. Our assays (since 1978) confirmed the theoretical background for these investigations: antibacterial, antihelminthic, deterrent, metatoxic and insecticidal characteristics were revealed. Cyanobacterial primary preparations were successfully used in laboratory and field testing as the agents for plant protection (Gol'din, 1995; Gol'din and Sirenko, 1998). Other results of last investigations also give evidences about the defensive role of cyanobacterial and microalgal secondary metabolites and their distinctions from toxic compounds affecting warm-blooded animals and a number of hydrobionts during water blooms and red tides (Macias *et al*., 2008).

The aim of our investigations includes two aspects: (1) evaluation of ecological and biochemical connections between cyanobacteria or microalgae and herbivorous organisms ("grazers"), and (2) formation of new approaches to the using of hydrobionts and their metabolites in human affairs. Here we give some of experimental data concerning the inhibition of vital functions of arthropods by cyanobacteria, their derivatives, and microalgae.

Materials and Methods

Cyanobacteria

Collection cultures were obtained from the Institute of Hydrobiology, Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (*Synechococcus leopoliensis* (Racib.) Komarek, *Nostoc muscorum* Ag., *N. linckia* Both., *Anabaena variabilis* Kütz., *Microcystis aeruginosa* Kütz. emend. Elenk.). Gorham and Zender's modification of Fitzgerald's medium – N 11 – was used for cultivation. Samples of natural material were collected in the Dnieper basin during traditional summer-autumn "water bloom" in collaboration with the Institute of Hydrobiology. In taxonomic structure of samples *M. aeruginosa* dominated (70.0-100.0%); besides *Microcystis* spp., *A. variabilis*, *Aphanizomenon flos-aquae* Ralfs ex Born et Flah., *Phormidium mucicola* Hub.-Pest. et Naum., and *Phormidium* spp. etc. occurred in samples. Cyanobacterial biomass was purified and treated by the way of thermal (37°C), lyophil or acetone drying and well-milled by laboratory grinding machines to get experimental samples of powder preparation. The aqueous suspension (0.5 and 1.0%) of experimental dry powder forms were used for the spraying of leaves intended for insect feeding.

Microalgae

Collection cultures of dinoflagellates (six species) were obtained from Institute of Biology of Southern Seas of the National Academy of Sciences of Ukraine: producers of toxins - *Kryptoperidinium foliaceum* (Stein) Lindemann (= *Glenodinium foliaceum* Stein.), *Lingulodinium polyedra* (Stein) Dodge (= *Gonyaulax polyedra* Stein.), *Gonyaulax* sp., *Prorocentrum micans* Ehr. (Hallegraef, 2003), and mass species – producers of biologically active substances and prey item for zooplankton *Gyrodinium fissum* (Lev.) Kof. et Sw. and *Gymnodinium kowalevskii* Pitz. (Pavlovskaya, 1969; Jeschke, 2002; Gol'din, 2008). Modified Goldberg medium was used for cultivation.

Culture

Conditions, extreme events and accumulation of biocidal compounds in the most active cyanobacteria and microalgae species were investigated.

Test-objects

In long-term laboratory and field investigations we conducted experimental exposures of lackey moth *Malacosoma neustria* L., brown-tale moth *Euproctis chrysorhaea* L., fall webworm *Hyphantria cunea* Drury and Colorado potato beetle *Leptinotarsa decemlineata* Say, at several life-history stages (eggs, different instar larvae and imago) to cyanobacteria and microalgae cultured strains, natural isolates and populations. Insects were taken from natural populations and kept in glass containers of 1.0 l volume, 10 specimens in each. Each variant of experiment included 5-10 replications. The leaves of the most typical host-plants (potato for Colorado potato beetle, apricot for lackey moth and brown-tale moth, and ash-leaved maple for fall webworm) were treated by cyanobacterial or microalgae culture via laboratory syringe and fed to target object. The observation included feeding behaviour, nutrition (% of consumed leave surface per individual), growth, metamorphosis, survival and histopathological examination of insects to control mechanism of biocidal activity.

Results

The results of research have scars to prove the different level of biocidal activity of cyanobacteria and microalgae (inhibition of a number of insect vital functions, including feeding, fat synthesis, growth, metamorphosis, reproduction, and as a result - lethal effect during all stages of life cycle and decrease of harmfulness), selectivity in dependence on cyanobacterial or microalgal species/culture (age, state, condition, fraction, nature of metabolites, biochemical composition, etc.), time of exposure, way of feed treatment, instar/phase of test object.

First of all, cyanobacteria and microalgae are possessed of general inhibitory effect to larval phases, especially in the stage of junior instars. At the same time after-effects were observed in posterior life cycle (inhibition of the processes of pupation, forming of imago and fecundity, and appearance of larval, pupal and imaginal teratogenesis).

The most important links of biocidal action include several components.

(1) Repellent activity is typical for *G. fissum* and *G. kowalevskii* in feeding experiments: lackey moth second instar larvae refused to feed treated leaves and concentrated in the substratum and walls of vessel; trophic reactions were absent during three-five days. The level of nutrition remained very low after replacement of treated food by normal diet, *e.g.* in the variant with *G. kowalevskii* the consumption was 2.5-3.0 times less than in control even in 7-10 days.

(2) Long-term deterrent activity was recorded for cyanobacterial natural populations, *S. leopoliensis*, *G. fissum* and *G. kowalevskii*: nutrition of Colorado potato beetle and lepidopterans larvae was high-grade inhibited – consumed leave surface was no more than 3.0-5.0% (dinoflagellates) or 1.5-42.8% (cyanobacteria) of control one (Table 1). Besides residual impact was observed during three-seven days after replacement of insects – nutrition was 0-30.0% to control.

(3) Short-term deterrent activity (suppression of feeding during first 24 h and follow-up consumption of treated leaves) was observed in *A. variabilis*, *N. muscorum*, *N. linckia*, *K. foliaceum*, *L. polyedra*, *Gonyaulax* sp. and *P. micans.*

(4) Inhibition of fat synthesis and growth processes: impact of cyanobacteria and dinoflagellates caused failures to thrive in second instar larvae, *e.g.* influence of *G. fissum* led to lag-effect in lackey moth (50.0%), fall webworm (29.2-68.0%), Colorado potato beetle (48.5%; some individuals – in 63.5%; imago – in 15.4-16.1%.); *K. foliaceum* - in lackey moth (50.0%), *G. kowalevskii* - in lackey moth (31.4%; some individuals – in 68.8%); *L. polyedra*, *G. fissum* and *G. kowalevskii* – in Colorado potato beetle (43.2%, 48.5% and 50.1%), natural cyanobacterial populations – in lackey moth (64.9-81.5%)), in fall webworm (20.0-30.7%), and in Colorado potato beetle (56.4-81.1%)

(5) Disorder of metamorphosis: the rates of pupation and imagination were decreased (Table 2). The imagination in Colorado potato beetle was 2.0-4.4 times less in assays with the natural cyanobacterial populations than in control one. The processes of pupation and imagination in lackey moth and fall webworm were accompanied by the significant morphological deflections, such as teratogenesis (non-viable individuals with bullate cuticle in the pupae of fall webworm and reduced wing covers in imago of Colorado potato beetle were observed in variants with a number of cyanobacterial samples). The treatment of eggs of Colorado potato beetle led to elimination of eggs and larvae in

Table 1. Deterrent activity of cyanobacterial biomass*

NN of samples	Ways of treatment of cyanobacterial biomass	Consumption of treated leaves during 10 days					
		Colorado potato beetle			Fall webworm		
		II instar	III instar	IV instar	II instar	III instar	IV instar
1	Roller drying	42.8±4.0	40.8±5.5	-	11.1±0.2	-	19.6±3.5
2	Lyophilization	18.7±3.2	55.0±7.5	-	1.5±0.2	2.0±0.5	42.0±7.7
	Thermal drying	18.0±4.6	49.8±7.0	74.4±3.2	0	12.0±2.6	32.2±10.6
	Aqueous filtrate of crude biomass (1:1)	7.1±2.2	35.7±6.1	79.1±1.9	12.0±1.0	29.5±9.7	32.5±8.3
3	Thermal drying	5.8±0.6	33.9±4.8	68.3±3.2	12.2±0.4	11.5±1.9	27.8±3.2
Control: water		100.0	100.0	100.0	100.0	100.0	100.0

* Leaves were treated by 0.5% suspension of powder; 150 larvae were placed in every variant, deterrent indices - the consumption of treated leaves during 10 days we give as % of control variant

Table 2. Cyanobacterial influence to the metamorphosis of fall webworm*

Cyanobacterial species	Metamorphosis of insects		
	III instar		IV instar
	Pupation	Imagination	Pupation
Synechococcus leopoliensis	14.7±1.3	8.0	13.3±6.8
Anabaena variabilis	33.3±2.1	9.3	0
Control	100.0	26.7	75.6±2.3

* 75 larvae in every variant; time of feeding by treated leaves – three days; pupation and imagination data are taken to the initial number of insects, %.

different variants - *G. fissum* (56.3% and 32.6%); *G. kowalevskii* (26.8% and 66.3%); *K. foliaceum* (0% and 9.3%); *L. polyedra* (3.4% and 34.3%); *Gonyaulax* sp. (6.1% и 27.3%) and *P. micans* (0.7% и 18.7%).

(6) Elimination: lethal effects were observed during 10-20 days (Tables 3-6). As a summary result, a probability of insect survival was very low.

Discussion

Experimental data confirm that biological and ecological constructiveness of mass and active cyanobacteria and microalgae species lays not only in toxic effect. It is complex, selective, multiple and many-sided challenge and a part of co-evolutionary process, which resides very close to allelochemical interactions between plants and herbivorous organisms in terrestrial ecosystems. Inhibitory effects maximally revealed in non-toxic dinoflagellates - food objects for herbivorous organisms in natural ecosystems, - compared to toxin producers; so toxic and non-toxic species have different activity to plant-feeders. Comparison and analysis of the indices of inhibitory processes in feeding, growth and metamorphosis and lethal termination in different variants of cyanobacterial samples can indicates the % of *M. aeruginosa* domination in blooming population as the determinant factor in the manifestation of biocidal activity.

Biocidal activity of cyanobacteria and microalgae is very similar to plant and microbial insecticides in their spectrum and mechanism of

Table 3. Mortality of the second instar larvae of Colorado potato beetle caused by cyanobacterial natural sample (thermal drying)

Concentration, %	Larval mortality, %		
	5 day	10 day	15 day
0.5	33.7±6.8	45.3±6.8	64.0±8.2
1.0	100.0	0	0
Control: water	0	5.3±2.7	5.3±2.7

* 75 larvae in every variant.

Table 4. Elimination of Colorado potato beetle, II instar larvae (mortality rates were accounted by Frantz equation)

Dinoflagellate species	Age of cultures (days)	Number of insects	Larval mortality (%)		
			10 day	15 day	20 day
Gyrodinium fissum	90	120	84.4±2.3	84.4±2.3	90.2±2.3
	60	45	93.3±4.5	100.0	
	90	120	15.0±4.6	35.8±2.3	35.8±2.3
Lingulodinium polyedra	90	75	34.6	34.6	34.6
Gonyaulax sp.	90	75	40.4	47.5	47.5

Table 5. Survival rates of lackey moth, II instar larvae (titer of cultures - 0.085 mln cells/ml).

Dinoflagellate species	Number of insects	Larval mortality (%)		
		10 day	15 day	20 day
Gymnodinium kowalevskii	75	4.3±2.7	26.5±10.9	96.4±3.4
Gyrodinium fissum	150	39.2±5.5	58.3±8.9	95.1±1.4
Kryptoperidinium foliaceum	75	0	0	0
Prorocentrum micans	75	4.1	12.5	49.2
Lingulodinium polyedra	75	5.5	11.0	49.5
Gonyaulax sp.	75	2.7	2.7	21.3
Goldberg medium	75	0	0	0

Table 6. Survival of fall webworm larvae (70 caterpillars in every variant)

Dinoflagellate species	Larval mortality, %						
	II instar			III instar			
	7 day	10 day	15 day	7 day	10 day	15 day	20 day
Gyrodinium fissum	82.2±6.8	97.8±6.8	100.0	52.9±11.3	77.1±16.2	88.6±9.7	100.0
Gymnodinium kowalevskii	53.3±4.5	84.4±11.4	97.8±2.3	35.7±3.8	55.7±7.7	88.9±4.1	100.0
Goldberg medium	4.4±2.3	6.7	15.6±2.3	0	0	2.2	2.2

action. It can be determined more likely as deterrent and inhibitory effect than toxic one, and cyanobacteria can be classed among selective and prophylactic agents.

Selectivity of biocidal effect of cyanobacteria and microalgae and high technological level of their controlled cultivation make possible to create new series of preparations for plant protection and pest control.

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