Stress Factors Enhancing Production of Algal Exudates: a Potential Self-Protective Mechanism?

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Algae are known to produce extracellular organic substances under optimum conditions and increase their production under stress. The changes in amount and composition of extracellular carbohydrates and proteins of three green algae *Scenedesmus quadricauda, Chlorella kessleri* and *Raphidocelis subcapitata* (known as *Selenastrum capricornutum)* were studied after a 5-days' cultivation under the influence of different types of stress factors (osmotic, organic, and heavy metal stressors). NaCl enhanced the quantity of carbohydrates more than proteins. A higher increase of proteins than carbohydrates was observed after addition of 3,5-dichlorophenol, glyphosate and cadmium chloride to algal cultures. The production of dissolved organic matter differs from species to species, with the age of a culture and the type of stressor.

Introduction

Bioavailability is one of the main factors that influence the toxic impact of a xenobiotic on an organism. The lipid solubility and the toxicant complexation with natural substances (humic acids, organic acids etc.) plays an important role in the membrane transport (Nelson and Donkin, 1985; Florence *et al.*, 1992). Organisms have evolved different self-protective mechanisms to maintain low intracellular concentrations of toxic substances: (a) active expulsion of toxicants after they have entered the cell, (b) complexation of metals by biologically synthesized ligands, and (c) oxidation, reduction, or chemical modification of the xenobiotics (Folsom *et al.*, 1986).

Algae are an important part of the water food chain and their ability to bioaccumulate or convert pollutants could play a considerable role in water management (Wong *et al.*, 1995). There is clear evidence that algae and cyanobacteria produce extracellular metabolites whose quantity as well as quality is dependent on nutritional status of algae, pH, light intensity, and presence of parasites (Sell and Overbeck, 1992; Maršálek *et al.*, 1992a; Maršálek *et al.*, 1992b).

The composition of algal extracellular products usually varies among species, according to nutrition and the physiological stage or life cycle of algae. Generally, organic acids, amino acids, peptides, sugars, poly- and oligosaccharides are present in the media after algal cultivation (Hellebust, 1974). The extracellular polypeptide of *Anabaena cylindrica*, for instance, forms complexes with various ions (Foog and Westlake, 1955). With a cell density of $2-4 \times 10^4$ cells/ml alga *Nitzia closterium* produces an exudate which can complex about 0.3 μM of Cu^{2+}. These exudates are produced only in response to copper ions and the excreted amount increases with concentration of copper (Lumsden and Florence, 1983). Vymazal (1987) observed a higher toxicity of Cd^{2+} than of Cu^{2+} for *Scenedesmus subspicatus,* and Kaplan *et al.* (1995) described the same effect for *Chlorella vulgaris* and *Chlorella saccharophila*. The observed differences in the sensitivity to these two metals may also be due to the differences in the production of metal-binding proteins, which are produced in response to various heavy metals and have been reported for various algae, for example *Chlorella spp.* (Rauser, 1990; Kaplan *et al.*, 1995).

The objective of this study is to determine the amount and composition of algal exudates under

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3,5-dichlorophenol and glyphosate stress (toxic organic compounds) in comparison with cadmium chloride (heavy metal) and NaCl (osmotic stressor).

Materials and Methods

Common planktonic algae *Scenedesmus quadricauda* (TURP) Breb. strain Greifswald 15, *Chlorella kessleri* (FOTT and NOVAK), *strain LARG 1.* and *Raphidocelis subcapitata,* known as *Selenastrum capricornutum*, strain SKULBERG, 1959/1 obtained from the Collection of Autotrophic Organisms at the Institute of Botany, Třeboň, were used. Cultures were cultivated in an orbital cultivator (6000 lx, photoperiod 12 h, 24°C, 1 litre of algal suspension in 2 litre bottles, continuous shaking, ISO medium (1989)). At the beginning of the exponential growth phase, stress factors had been applied for 2 days as follows: NaCl 0.6M, $CdCl_2$ 0.03 μM, 3,5-dichlorophenol 0.4 M, glyphosate 0.2 M solutions. The algal cultures were harvested at a density of $2x10^6$ cells/ml by centrifugation and the supernatant was used for determination of algal exudates. The total amount of carbohydrates was determined by the phenol-sulphuric method (Šafařík and Šantrůčková, 1992). The total amount of proteins was determined spectrophotometrically at 545 nm using the method with Coomasie blue (Bradford, 1976). Sugars and amino acids were separated after acid hydrolysis on HPTLC plates and determined by the scanning densitometer (Shimazu, Japan; PAG, 1986).

The statistical differences in the production of exudates between controls and stressed algal cultures were evaluated by the non-parametric Wilcoxon test, α=0,1 (Cohran and Cox, 1957). The results come from the mean of three replicates for every experimental variant. If the variability among the replicates exceeded 10% the experiment was repeated.

Results and Discussion

The results of the experiments with three different species of algae *Scenedesmus quadricauda, Chlorella kessleri* and *Raphidocelis subcapitata*, show that the production of extracellular substances can vary from species to species. However, generally the amount of extracellular proteins and carbohydrates increased significantly under the stress conditions (Table I). However, these results are from the young cultures (in the middle of the exponential growth phase). The cultures at the end of the active growth may contain a 3–12 times higher amount of exudates (Maršálek *et al.*, 1992a).

The composition of carbohydrates as well as proteins was different under the influence of different stressors but both types of exudates showed similar quantitative responses among investigated species. The exposure to NaCl resulted in a con-

Table I. The composition of selected compounds in the medium after the cultivation of algae. All values are in μg/l and represent average of three replicates. A = *Scenedesmus quadricauda,* B = *Chlorella kessleri,* C = *Raphidocelis subcapitata.*

	Control			NaCl			Dichlorophenol			Glyphosate			$CdCl_2$		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Total proteins	619	729	506	969	1163	880	2315	1881	1626	2816	1966	1815	3595	2863	2356
Total carbohydrates	215	327	188	1865	1936	1126	617	957	670	896	1051	821	581	698	496
Glycine	23	32	38	28	61	41	31	96	63	29	81	113	36	101	63
Cysteine	29	41	44	28	59	56	62	103	71	58	122	123	76	133	97
Glutamine	12	33	16	13	46	37	17	91	61	17	83	88	19	73	71
Glutamic acid	24	23	27	27	47	63	68	83	83	51	113	96	79	131	101
Proline	16	27	18	68	86	102	75	93	95	71	137	127	79	126	117
Xylose	25	33	41	215	236	271	81	111	103	58	75	73	75	80	66
Galactose	17	28	28	261	241	247	96	106	816	67	73	65	63	77	71
Glucose	19	25	19	161	280	118	39	103	67	42	70	37	47	80	57
Arabinose	26	31	33	126	268	252	58	118	92	56	61	48	47	63	67

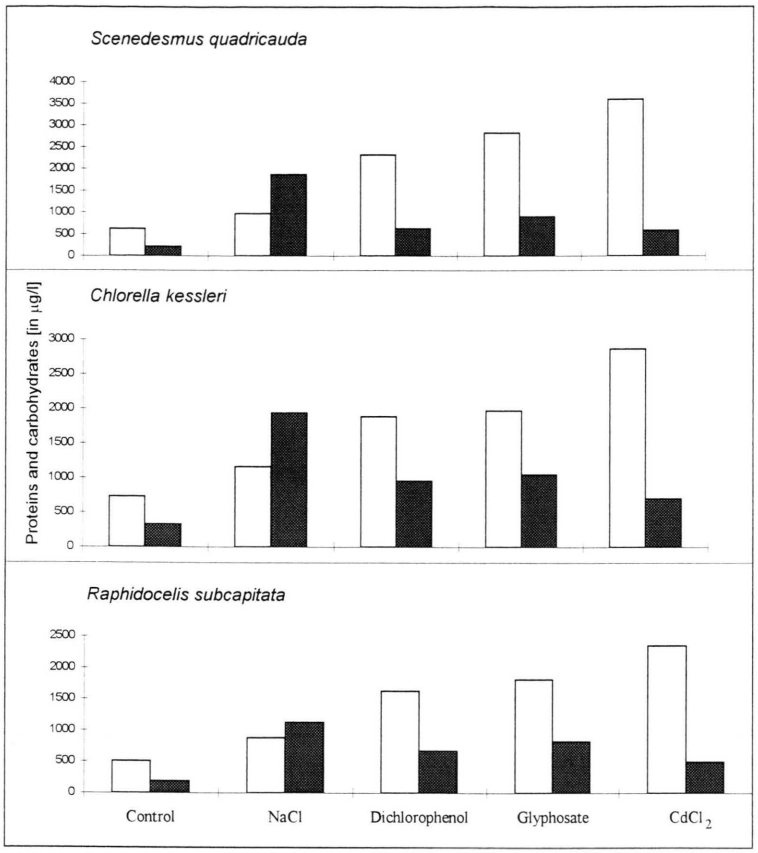

Fig. 1. The amount of total extracellular proteins and carbohydrates in the medium after the 48-h cultivation of algae under stress. □ Total proteins, ■ Total carbohydrates.

siderable increase of total carbohydrates, whereas the total protein content was affected only slightly (Fig. 1). On the contrary, the production of extracellular proteins was more enhanced after addition of the toxic organic compounds (dichlorophenol and glyphosate) and cadmium. The lowest amount of carbohydrates was produced during cadmium exposure, while the same stress factor led to the highest excretion of proteins of each alga. Thompson and Couture (1991) observed in their study that *Selenastrum capricornutum* (*Raphidocelis subcapitata* in our study) produces three times more proteins than carbohydrates when exposed to cadmium ions. In our case this ratio was much higher (nearly 5 times).

The results presented in the tables are similar to the data of Eberhard and Wegmann (1989) who reported that algae increased the production of proline under stress. However, they focused their attention on the cellular content under cold or drought stress. Wu *et al.* (1995) supported the idea that proline is a compound accumulated in algal cells as a osmoprotectant in relation to the level of salinity, osmolarity and drought as well as to the high levels of intracellular metals. Their experiments showed that a more tolerant species to copper or cadmium accumulates more proline than a sensitive one does. An experiment conducted with a supply of exogenous proline indicated that proline can significantly lower the toxicity of copper to *Anacystis nidulans*.

According to our experience, the quantity of compounds released from algal cells depends on the physiological stage of cultures and thus the algal culture should not be expressed generally as a number of cells per ml. Frequently, a higher amount of exudates per cell is released from cultures at the beginning of exponential growth phase than during the stationary phase. The intensity and type of stress or the physiological stage of the cultures seem to be the main factors increasing the extracellular algal production.

As mentioned above the intracellular accumulation of algal metabolic products plays also an important role in the protection of algae against the stress factors (Kaplan *et al.*, 1995). One of the most widely spread metal detoxicants are polypeptides, phytochelatins, which were isolated not only from algae but from higher plants and fungi as well (Ahner *et al.*, 1995; Ahner and Morel, 1995).

The natural phytoplankton populations are also discussed as the producers of the algal extracellular substances. There exists a recently published opinion proposing that a reason for algal exudation is to reduce virus infection by encouraging bacterial growth, which in turn may support flagellates. This theory is based on the fact that phytoplankton cells exude dissolved organic matter that supports growth of local bacterial community and consequently heterotrophic flagellates. Both bacteria and flagellates remove more than 50% of viruses before they have a chance to infect their host phytoplankton cell (Murray, 1995). The fact that extracellular products of phytoplankton are utilized by heterotrophic bacteria has been known for a long time. For example, this phenomenon was observed in algal blooms that have higher exudation rates and are closely associated with bacterial development (Nalewajko *et al.*, 1980). However, the relationship is more complicated when one considers that the significance of phytoplankton extracellular products to bacteria differs among environments and time, moreover, the nutritional relationship between algae and bacteria is influenced by zooplankton. In addition, bacteria themselves lyses algal cells and their number increase after the collapse of algal population. On the contrary, they can be suppressed by antibacterial substances produced by algae. Nevertheless, the antiviral protection can be one of the reasons for exudation.

Three algae, whose extracellular production is documented in this paper, are frequently used as test organisms for toxicity assessment (ISO, OECD, U.S.EPA). In connection with the discussed extra- and intracellular products it is an open question whether an algal organism can influence the impact of a toxicant during an algal assay period and change its sensitivity. This phenomenon will be investigated further in a more detailed study.

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