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Antimutagenic Properties of Fresh-Water Blue-Green Algae

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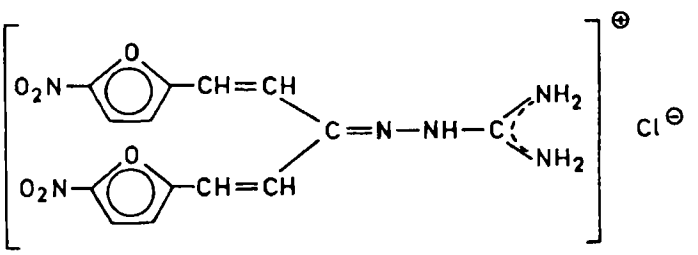
ABSTRACT. The antimutagenic properties of whole fresh-water blue-green algae *Aphanisomenon flos-aquae*, marketed under the commercial name "Alpha Sun" were tested using the Ames test. Simultaneous addition of both algae and Nitrovin (a mutagen) to the test medium did not reduce the mutagenic activity. On the other hand, addition of freeze-dried blue-green algae to the test medium 2 - 24 h before the application of mutagen reduced its mutagenic activity.

It is well-known that the mutagenic properties of chemical compounds follow closely their cancerogenic activity. On the other hand, however, substances possessing chemopreventive properties against cancer often exhibit also antimutagenic properties. Since the cancer-preventing ability of various algae is known (Reddy *et al.* 1984; Yamamoto *et al.* 1982, 1986; for review see Hocman 1989), it appears to be useful to test the possible antimutagenic properties of whole blue-green and other fresh-water algae in the Ames test.

MATERIAL AND METHODS

Blue-green and other fresh-water algae (*Aphanisomenon flos-aquae*, *Spirulina* sp., *Chlorella* sp.) grown in the Upper Klamath Lake (Oregon) were mechanically cleaned. Whole, native, blue-green algae frozen to −27 °C, were subsequently freeze-dried. Such treatment ensures the preservation of all biologically active, labile substances, such as enzymes, vitamins and chlorophyll. The main portion (over 95 %) of the mixture of algae consisted of *Aphanisomenon flos-aquae*, others are present in smaller quantities only. This product is marketed under the name "Alpha Sun", by *Cell Tech*, Klamath Falls (Oregon, USA). The freeze-dried algae were dispersed in water by short sonication.

The test of antimutagenicity was done by the Ames technique on *Salmonella typhimurium* strains TA97, TA100, TA102 (Maron and Ames 1983). Nitrovin (1,5-bis(5-nitro-2-furyl)-1,4-pentadiene-3-one-iminosemicarbazone hydrochloride, purchased from *Chemapol*, former Czechoslovakia) was used as mutagen at concentrations indicated in Table I. The mutagen was added to the test medium dissolved in dimethyl sulfoxide. The number of revertants produced by this mutagen was considered to be 100 % at each concentration of nitrovin. The addition of tested antimutagens resulted in lowering the number of revertants, again expressed as percentage against the number of revertants appearing under the influence of the mutagen alone. The lower this value the more intense the antimutagenic efficiency of the compound tested. The tests were done in triplicate. The numbers of revertants (Table I) are means of these values.



Nitrovin

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Table I. Ames' test performed with the mutagen Nitrovin (N) and algae (A)[a]

N	A	TA97		TA100		TA102	
μg per plate	mg per plate	*n*	%	*n*	%	*n*	%
Nitrovin and algae put simultaneously							
0.5	0	1 173	100	1 185	100	–	–
	0.125	1 202	102	1 094	92	–	–
	0.25	1 255	106	1 173	99	–	–
	0.5	1 265	107	1 177	99	–	–
	1	1 248	106	1 254	106	–	–
	2	1 231	105	1 166	98	–	–
Algae present 2 h before the addition of Nitrovin							
1	0	794	100	665	100	623	100
	0.25	696	87	677	101	–	–
	0.5	634	79	716	107	358	57
	1	749	94	688	103	–	–
0[b]	0	118		121		138	
	0.5	–		–		126	
	1	132		186		–	
Algae present 24 h before the addition of Nitrovin							
0.5	0	1 030	100	665	100	326	100
	0.25	–	–	400	60	–	–
	0.5	–	–	387	58	–	–
	1	–	–	349	52	–	–
	2	521	50	–	–	128	39
1.0	0	972	100	–	–	445	100
	2	532	55	–	–	157	35
0[b]	0	106		121		139	
	1	–		186		–	
	2	85		–		32	

[a]At 37 °C. Numbers of revertants (*n*) are means of three measurements on each strain of *S. typhimurium* (TA97, TA100, TA102).

[b]Control (numbers of spontaneous revertants are given).

Several concentrations of Nitrovin as well as that of algae were tested on each strain of *S. typhimurium*. Since we intended to test the preventive antimutagenic properties, the algae were added to the test medium not only simultaneously with the mutagen, but also 2 and 24 h before the addition of Nitrovin. The test mixture of *Salmonella* in nutrient broth and the algae were kept at 37 °C throughout.

RESULTS AND DISCUSSION

The results in Table I show the numbers of revertants in the Ames test where both Nitrovin and algae were introduced into the test medium simultaneously. The results indicate that no decrease in the mutagenic activity of Nitrovin occurred.

Blue-green algae present in the test medium at least two hours before the addition of the mutagen caused a decrease of mutagenic activity. This antimutagenic action was marked in strain TA102 but no such effect was present in strain TA97. The presence of algae alone in the test medium

at concentrations higher than 2 mg per plate showed an inhibitory effect on the growth of *Salmonella* strains.

However, the suppression of mutagenic activity after 2 h of the presence of algae was not clear. Apparently, the different results with various strains of *S. typhimurium* and differences in antimutagenic activity connected with various concentrations of both algae and Nitrovin led to such results. We describe these differences to the short duration of the influence of antimutagenic algae. More impressive results were obtained when the algae were allowed to exert their influence on the tester strains for 24 h at 37 °C (Table I).

It could, therefore, be concluded that

(*1*) the fresh-water blue-green algae ("Alpha Sun") do not exert any antimutagenic activity when added to the Ames test mixture simultaneously with the mutagen Nitrovin;

(*2*) the blue-green algae present in the test medium together with the *Salmonella* strains for 2 or 24 h exhibit a marked antimutagenic action decreasing the number of revertants. The most intense suppression of mutagenic activity has been achieved when the algae were present for 24 h before the addition of the mutagen;

(*3*) different strains of *S. typhimurium* react in different ways to the mutagen application in the presence of blue-green algae;

(*4*) in some cases the blue-green algae caused marked lowering of the numbers of spontaneous revertants;

(*5*) the blue-green algae themselves did not exert any mutagenic effect whatsoever in the Ames test.

These results are only preliminary, owing to the different time intervals, concentrations and strains used in each of the experiments, and with only one mutagen. However, we consider the antimutagenic properties of blue-green algae ("Alpha Sun") as established when they are present in the test medium for some time before the application of the mutagen.

These experiments have been made possible by samples of "Super blue-green algae Alpha Sun", kindly donated by *Cell Tech*, Klamath Falls (Oregon, USA).

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