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New Toxicity Testing Method Using Marine Bacterivorous Nanoflagellates

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A toxicity test with marine test organisms is useful for substances, who›s future application is the marine environment (Henke 1986). Here a method is described which deals with marine organisms, who play an important part in the marine ecosystem: the bacterivorous flagellates. Several investigations of the last decade verified their significant role in marine food webs as a link between bacterial degradation of organic material and animals of higher trophic levels (Fenchel 1982). That is the reason, why it seemed correct to use these organisms for toxicological studies. The presented method is a modified growth inhibition test with similar sensitivity as other tests and a easy to evualate.

The test is based on the fact, that bacteria required for the growth of bacterivorous flagellates are less sensitive to low concentrations of chemicals, as previously described by Bringmann and Kühn (1970). A small population of about 100 flagellates per mL grows into a dense population of $\geq 10^6$-10^7/mL, if a sufficient ("threshold") concentration of bacteria of about 10^7/mL is present. Such a bacterial titer arises, when a moderate but sufficient growth of bacteria is stimulated by supplementation with an easy degradable C/N-source. A dense flagellate population of $\geq 10^5$/mL is easyly detected by microscopic examination in a counting chamber (2 per single counting square). Moreover, in most cases such a dense flagellate population is visible by the clearance of the water. The propagation of such a dense flagellate population can be inhibited by toxicant concentrations, which are too small for hindering of bacterial growth but sufficient for flagellate inhibition.

MATERIALS AND METHODS

A mixed population of marine bacterivorous nanoflagellates were obtained by collecting 500 mL seawater from the station "Cablebouy, Helgoland, FRG" along with food organisms (bacteria etc.).

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The sample will be cultured at 18° C in the dark on a shaker until it had grown upto a concentration of 10 flagellates per mL using a supplementation of 0.5 g of peptone per liter of seawater. After 1 day a concentrated bacterial population was visible by its optical density (OD), and after 1-2 more days the flagellate grazing had cleared the bacterial population. Such water contained about 10^7 flagellates per mL. This enrichment culture was diluted down to a concentration of 1000 flagellates as stock culture. Glass bottles (20 mL volume, filled with 9 mL test medium: 0.5 g peptone per liter seawater and a fixed concentration of toxicant) were inoculated with 1 mL flagellate stock culture and cultured at 18° C in the dark on a shaker (starting population: 100 flagellates and an unknown number of bacteria per mL).

Each day the cultures were examinated by phase-contrast-microscopy at 400x magnification for flagellates growth using a Petroff-Hausser counting chamber (C.A.Hausser & Sons., Phila., USA). The tested toxicant concentration was judged nontoxic when $\geq$ 2 flagellates per counting square ($\geq$ 5 x 10 mL of culture) were present in the counting chamber while cultures with smaller flagellate content were judged toxic.

The method presented was used for several toxicity testings and the results were compared with the bioluminescence inhibition of Photobacterium phosphoreum (Microtox test, carrying-out as described previously by Krebs 1983) and the mortality test of the brine shrimp Artemia sp. (method described by Henke 1987). Heavy metals, phenol, and surfactants were selected as test substances because toxicity values of most of them derived from the test systems listed above were available.

RESULTS AND DISCUSSION

In most cases the control series without toxicant reached the dense flagellate level of $\geq 10^5$/mL after 2 days representing a growth period of 0.29 d. Cultures with increasing concentrations of toxicants showed increasing delays to reach this dense concentration, but most series with untoxic concentrations of the tested substances reached it not later than 5 days. The limit of 7 days representing an enlarged multiplication rate of 0.71 d was not found at any of the test series. Therefore, it seemed correct to use this 7 d limit for the final control of the cultures and to judge the enlargement of the generation time over 0.71 d as toxic effect.

The comparision of the test system presented here with other systems dealing with marine test organisms is listed in table 1. The most sensitive test was the bioluminescence test (exception: CTAB). Unfortunately, the disadvantage of this test is the missing measurment of a clearly toxic effect, because bioluminescence reduction is definitely not similar with "illness" of the organism (Lümmen 1988). Nevertheless, bioluminescence inhibition is very sensitive and gives similar toxicity ran-

Table 1. Toxicity data from 3 test methods with marine test organisms; $EC_{fla-tox}$ = toxic concentration range against flagellates, EC_{50} = concentration of 50% luminescence inhibition, $LD_{art-tox}$ = letal dosis for 50% tested _Artemia_; tested surfactants are TL-2 = trehalose-dicorynomycolate, CTAB = cetyltrimethyl-ammoniumbromide, E09 = nonylphenol-ethoxylate$_9$, E04.5 = nonylphenol-ethoxylate4.5, Pril = cleaning surfactant, Corexit and Finasol = oil dispersants

	Testorganism:		
	Nanoflagellates mixed population	Bakteria _P. phosph._	Shrimp larvae _Artemia sp._
Test-substance	$EC_{fla-tox}$ (ppm)	EC_{50} (ppm)	$LD_{art-tox}$ (ppm)
Heavy metals			
$ZnSO_4 \cdot 7\ H_2O$	23 - 115	0.43	
$CuSO_4 \cdot 5\ H_2O$	13 - 19	0.08	
Phenol	500 - 1000	26	
Surfactants			
TL-2	500 - 1000	63	10000
CTAB	1 - 5	86	
E09	60 - 80	78	
E04.5	15 - 20	79	
Pril	10 - 50	35	10
Corexit 9527	50 - 100	5	162
Finasol OSR5	13 - 50	7	34

kings as other methods (Liu and Dutka 1987). The LD_{50}-values of the mortality of the brine shrimp _Artemia_ are in the same range as the $EC_{fla-tox}$-data (exception: TL-2).

The toxicity test described is a useful completion of the known methods. It measures a clearly toxic effect on an important organism of the ecosystem. A significant advantage of the test procedure is its easy realization and no requirements of expensive instruments. Moreover, there is hardly no need for sterile working, because the presence of bacteria in the test medium is necessary for the propagation of prey organisms.

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