Bull. Environ. Contam. Toxicol. (1988) 40:18–22

Bulletin of Environmental Contamination and Toxicology

Comparison of Antibiotic Production from Four Ecotypes of the Marine Alga, *Dunaliella*

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Coastal waters provide an environment which is abundantly rich in nutrients and biomass. The environmental pressures on these regions are particularly intense. Many pollutants, including human sewage and industrial wastes, are added to coastal waters providing nutrients and increasing the microbial population. One mechanism for species survival in a highly competitive situation is the release of bioactive metabolites. It is a stratagem to facilitate survival and overcome competition found in terrestrial and marine vegetation (Norris and Fenical 1982).

Since the pioneering work of Pratt (1942) on the antibacterial activity of chlorellin produced by Chlorella, many researchers have studied the antibiotic activity of substances produced by phytoplankton. These secondary metabolites include unique fatty acids, halogenated compounds and pigment derivatives (Sieburth 1959, Trick et al. 1984, Mason et al. 1982, Bruce and Duff 1967, Gauthier et al. 1978). In a study of a wide spectrum of planktonic algae, Duff et al. (1966) found the greatest antimicrobial activity among the Bacillariophyceae, Chrysophyceae and Cryptophyceae. While most green algae are poor producers of antimicrobials, a survey of the Volvocaceae showed that this family contains members which produce a non-protein, heat labile substance that inhibits the growth of other planktonic algae (Harris 1971, Harris and Parekh 1974).

The present study was undertaken to examine the production of an antibiotic substance from four strains of the marine alga, Dunaliella, two from waters of high pollution and two from waters of low pollution.

MATERIALS AND METHODS.

Dunaliella is a unicellular green flagellate of the Order Volvocales. The ecotypes studied were D. salina isolated from San Francisco Bay, CA (DSA), from Great Salt Lake, Utah (DSU), and from Long Island Sound, NY (DSL). D. bardawil was isolated from the Sinai, Egypt (DB). They were grown in 250ml shake flasks, containing 150ml of artificial sea water medium.

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modified DC medium (Provasoli et al. 1957), at 22°C with continuous light of 1500 lux in an environmental chamber. Cultures were harvested at 7, 14, 21 and 28 days. It was found that the greatest activity was produced at 21 days, which corresponded to late log phase.

Cultures were centrifuged at 1500 Xg for 10 minutes. The pellet of cells was lysed with 20ml of distilled water and examined with a microscope for lysis. Both supernatant and lysed cells were tested for antibiotic production by challenge against a series of gram (+) and (-) organisms, Mycobacterium smegmatis and Saccharomyces cerevisiae. Each culture was tested four times.

In the additional research, DSA was used since this isolated strain showed the greatest activity. The pellet was prepared as before, then treated with 50ml of the following solvents to determine solubility: methanol, ethanol, propanol, butanol, ethyl acetate, water, chloroform. Effectiveness was measured by immersing filter paper discs into the solvent systems. The disks were then removed and placed in sterile glass petri dishes to dry at 4°C in the dark. The length of time for drying varied from 24 hours to 4 days dependent on the solvent. Solvent controls were used. The control discs were immersed in the solvent chemical then left to dry for the same time period as the experimental discs. After being thoroughly dry, the discs were placed on lawns of the test bacteria. Zones of inhibition were measured at 24 hrs. Each solvent was tested a minimum of four times.

To measure the effect of temperature, the pellet of cells was lysed in distilled water as before. Five ml samples were exposed to 4, 25, 37 or 45°C for 1 hr. or 24 hrs. The lysed cells were then added to wells made in agar plates that were seeded with bacteria. Zones of inhibition were measured at 24 hrs. The procedure was repeated four times.

To measure the effect of pH, the pellet was treated as above. The 5ml samples were exposed to pH 4, 7, 9, 11 for 1 hr. or 24 hrs. After the allotted time, the sample was brought to pH 7. The sample was then added to wells and treated as described above. The procedure was repeated four times.

Pellets were exposed to 0.5 mg/ml of DNAse RNAse, protease or 1000 IU/ml penicillinase for 24 hrs. at 22°C. The sample was then added to wells and treated as above. This procedure was repeated four times.

RESULTS AND DISCUSSION.

The results of this study indicate that a broad spectrum antibiotic substance is produced by two of the isolated Dunaliella cultures, DSA and DSL. These cultures were isolated from waters of high pollution. No zones were seen with DB or DSU from waters of low pollution (Table 1). Lysed cells were more active than the supernatant. Figure 1 shows the zone produced by lysed DSA.

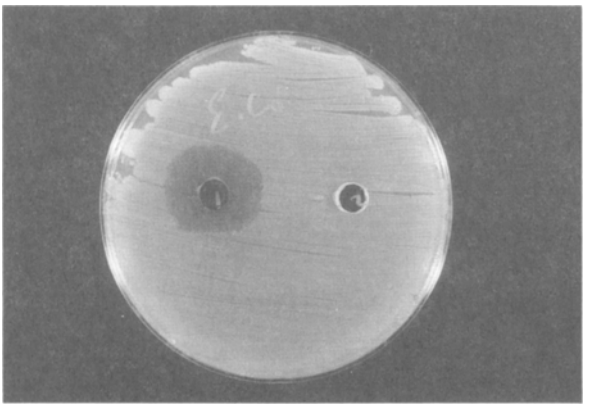

Figure 1. Zone of inhibition produced by lysed DSA cells (0.1 ml) with Escherichia coli. 1. DSA 2. Sea water medium

Table 1. Zones of inhibition produced by Dunaliella salina (mm)

Organism	DSA		DSL	
	Lysed cells	Supernatant	Lysed cells	Supernatant
E. coli	15±2.20	13±1.40	13±3.47	12±2.38
P. aeruginosa	16±3.25	12±2.36	14±3.16	12±3.44
P. vulgaris	15±2.57	10±3.84	14±1.85	13±3.61
S. gallineum	16±3.34	11±2.71	13±2.36	11±2.17
S. epidermidis	13±1.79	12±1.62	11±1.92	10±3.43
M. luteus	10±2.63	10±2.45	11±1.74	10±2.68
B. subtilis	-	-	-	-
M. smegmatis	-	-	-	-
S. cerevisiae	-	-	-	-

No zones with D. bardawil, D. salina from Great Salt Lake, Utah.

Table 2. Solvent activity

Organism	H_2O	Acetone	Ethyl Acetate	Propanol	Butanol
E. coli	16±1.2	-	12±0.8	18±2.4	20±2.5
S. gallineum	17±0.8	12±0.6	15±1.1	-	16±1.4
M. luteus	10±0.5	12±0.7	10±0.7	-	13±0.9
P. vulgaris	-	-	18±2.3	18±1.5	17±1.1
P. aeruginosa	-	-	12±1.2	23±1.2	25±3.3
S. epidermidis	15±1.4	11±0.9	13±1.3	17±1.0	19±2.4
B. subtilis	-	-	-	-	-

Solvents methanol, ethanol, and chloroform showed no activity.

Solvent activity showed that the largest alcohol, butanol, had the largest zone of inhibition (Table 2). Ethyl acetate was next. Propanol and water were also active. The smaller alcohols, methanol, and ethanol had no activity, nor did chloroform.

Table 3. Effect of temperature on activity of lysed cells of DSA

1 Hr. Organism	4^0	25^0	37^0	45^0
E. coli	13.9±1.57	14.0±1.29	9.6±6.73	-
P. aeruginosa	13.1±1.35	14.0±1.41	3.5±6.50	-
P. vulgaris	-	-	-	-
S. epidermidis	11.9±1.14	12.0±1.79	6.2±5.95	-
M. luteus	13.7±1.70	14.3±2.29	7.4±7.23	-
B. subtilis	-	-	-	-
24 Hr.				
E. coli	13.4±1.62	14.0±1.38	7.3±6.92	-
P. aeruginosa	10.2±6.06	10.6±6.07	5.2±8.06	-
P. vulgaris	-	-	-	-
S. epidermidis	12.7±1.38	13.3±1.80	6.7±6.32	-
M. luteus	13.0±1.03	13.6±2.23	5.1±6.59	-
B. subtilis	-	-	-	-

The substance is inactivated by uv light. It is heat labile (Table 3). Activity was destroyed by 45°C within 1 hour and there was decreased activity at 37°C. It was inactivated by acid pH 4, but alkaline pH 11, did not decrease antibiotic activity (Table 4). Enzymes for DNA, RNA, protein or penicillin were not effective in destroying activity indicating that the substance is a heat-labile non-protein material.

Table 4. Effect of pH on activity of lysed cells of DSA

1 Hr. Organism	4	7	9	11
E. coli	-	13.2±1.30	13.0±0.71	14.0±1.22
P. aeruginosa	-	12.4±0.89	14.0±1.00	14.2±1.30
P. vulgaris	-	-	-	-
S. epidermidis	-	11.7±1.37	12.3±1.51	14.0±0.63
M. luteus	-	13.7±1.03	14.5±1.38	14.8±0.98
B. subtilis	-	-	-	-
24 Hr.				
E. coli	-	12.8±1.33	14.0±0.63	14.8±1.60
P. aeruginosa	-	13.8±1.33	14.2±0.75	15.2±0.41
P. vulgaris	-	-	-	-
S. epidermidis	-	12.0±1.10	13.8±0.75	14.5±1.38
M. luteus	-	13.6±1.14	13.6±0.69	15.2±0.84
B. subtilis	-	-	-	-

The antibiotic substance is produced by those strains of Dunaliella which were isolated from waters of high pollution, but not from those of low pollution. The active strains flourish in environments which contain numerous bacteria and other microorganisms. Forms which can survive in a stressed environment need to have some adaptive advantage to allow for their growth. The release of antibiotic or allelopathic chemicals into seawater will give an adaptive advantage to the releasing organism (Barbier 1981). It will alter the microenvironment surrounding the releaser and provide it with enhanced space and sunlight. The release of bioactive metabolites represents one mechanism of species survival in a highly competitive situation.

Acknowledgments. This research was supported by a mini-grant from the New Jersey Marine Sciences Consortium, Ft. Hancock, NJ.

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Received July 31, 1987; accepted September 28, 1987.