Offprint from "Marine Biology"
International Journal on Life in Oceans and Coastal Waters, Vol. 12, No. 3, February 1972, Pages 189—193

Avec les hommages des auteurs

Are some bacteria toxic for marine algae?

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Abstract

It was observed *in vitro* that some bacteria from different origins, including the sea, are capable of inhibiting the growth of several marine algae. However, there is too much difference between *in vitro* experiments and *in situ* environmental conditions to assume that bacterial poisons may rule a great deal of algae-bacteria relationships in the sea as a whole. In the author's opinion the importance of bacterial poison is limited to special and rare instances.

Introduction

There is now a great deal of evidence that the algal and bacterial environment in the sea is not only ruled by a simple trophic relationship; antibiosis may also play an important role in marine ecology.

Red-tide algal toxins have attracted attention over a long period because of their toxic effects on fishes and even mammals, including man (review by Shilo, 1967). Some marine algae have also been proved capable of antibiotic synthesis against bacteria (Steeman-Nielsen, 1955; Sieburth, 1959; Burkholder et al., 1960; Aubert, 1965; Duff et al., 1966).

It has long been recognised that sea water possesses bactericidal properties. Rosenfeld and Zobell (1947) suggest that this may be due to the existence of an autochthonous bacterial microflora which produces antibiotic substances. Krasil'nikova (1964), Burkholder et al. (1966) and Gauthier (1969, 1970) report the production of antibacterial substances by marine bacteria.

In regard to organisms isolated from both fish and waste ponds, Shilo (1967) and Stewart and Brown (1969) pointed out that, in mixed-plate cultures, the bacteria may lyse Cyanophyta or Chlorophyta cells.

In our *in vitro* experiments we observed that the substances released by some bacteria inhibit marine algae growth until death ensues.

Material

The following algae species were used. Chlorophyceae: *Chlamydomonas magnusii*[1]; Prasinophyceae: *Prasinocladus marinus*[2], *Tetraselmis striata*; Bacillariophyceae: *Amphora* sp., *Asterionella japonica*, *Chaetoceros lauderi*, *Hemiaulus sinensis*, *Nitzschia acicularis*, *Phaeodactylum tricornutum*, *Thalassiosira rotula*; Chrysophyceae: *Stichochrysis immobilis*[1]; Xanthophyceae: *Heterothrix* sp.[3], *Monallantus salina*[1].

All strains were previously obtained in bacteria-free cultures and routinely grown in Provasoli's (1968) ES solution. *Tetraselmis striata* and *Nitzschia acicularis* are Provasoli's collection strains (Haskins Laboratories, New-Haven, USA) obtained through the courtesy of Dr. Aubert (C.E.R.B.O.M., Nice, France); the other species were isolated from the Gulf of Marseilles.

Several of the bacterial strains which have been tested were obtained from the Institut Pasteur (Paris, France) by courtesy of Dr. Bianchi (Station marine d'Endoume): *Pseudomonas aeruginosa* (C.I.P. strain number 6352), *P. fluorescens* (5690), *P. maltophilia* (5490), *P. putida* (6323), *Vibrio cholerae* (6215). Dr. Bianchi also provided strain 558, which is a *Pseudomonas* very closely related to *Pseudomonas pseudoalcaligenes*, and strain 601, a brevibacterium; both were collected from deep mud (Bianchi, 1971). Twenty strains were isolated from algae cultures as previously described (Berland et al., 1969); 8 *Pseudomonas*, 2 *Vibrio*, 4 *Achromobacter* and 6 *Flavobacteria* were used. Three other strains of the same origin, as yet undetermined, were also used (reference numbers 00.27, 00.29, 00.30 of our collection). Two strains (references 26.32 and 26.51) were isolated off the Gulf of Fos, near Marseille. These 5 strains are presently being investigated as to their taxonomic position.

Toxicity of *Pseudomonas aeruginosa* to *Tetraselmis striata*

Pseudomonads are frequently reported to cause diseases in animals and plants (Shackleton, 1968; Sinden and Durbin, 1969; Heckley, 1970); we attempted to discover if they are also toxic to marine algae. *Pseudomonas aeruginosa* was chosen, both

[1] Determined by Professor P. Gayral, Caen.

[2] Determined by Dr. M. J. Chretiennot, Marseille.

[3] Determined by Dr. P. Bourrelly, Paris.

because of its wide occurrence (it is possibly run off with sewage to the marine environment), and because it is a toxic pigment-synthetizer, which indicates when toxic substances appear. The motile chlorophyta *Tetraselmis striata* was used as a test strain (motility indicates the physiological state of the cells), and cultivated in ES solution prepared from supernatants of *Pseudomonas aeruginosa* cultures, centrifuged at different steps of development.

Methods

(1) The bacteria were grown at 18 °C in enriched sea water containing bacto peptone: 2 g; beef extract: 0.5 g; yeast extract: 0.5 g; 80 % sea water: 1 l; pH: 7.5 to 7,8; sterilization: autoclaving (20 mn at 120 °C).

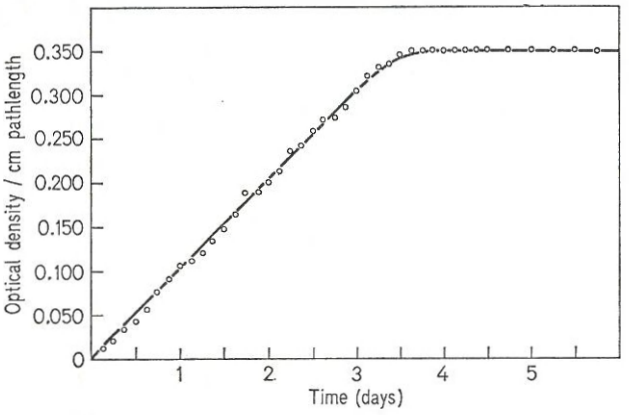

Fig. 1. *Pseudomonas aeruginosa*. Growth curve, expressed in optical density/cm pathlength of cultures

This medium allows pigment synthesis of *Pseudomonas aeruginosa*; it appears when the optical density (OD) of the culture is around 0.075/cm path-length (Fig. 1). The culture vessel was a 40 mm path-length spectrophotometric tube, containing 100 ml medium. Inocula were young (24 h old). Growth was followed by measuring the OD of the culture, using a SAFAS spectrophotometer; readings were made at 600 nm.

(2) The cultures were centrifuged and cells removed. Then ES nutrients were added to the supernatants in order to prepare a Provasoli's ES solution for algae cultures. Sterilization was conducted by filtering through a Swinnex Millipore filter holder, containing one GS 0.22 µ Millipore filter. One hundred ml filtrate-ES solution were poured into a sterile 40 mm-diameter spectrophotometric cuvette.

(3) A bacteria-free culture of *Tetraselmis striata* was inoculated into the tubes and incubated at 18 °C. The algal growth was followed by measuring four times a day the OD at 600 nm. A special arrangement of

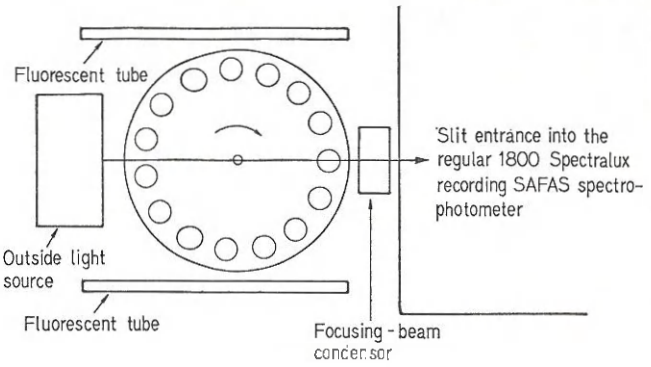

Fig. 2. Growth recording equipment. Extra light source located outside the usual apparatus; light beam focussed on slit entrance by accurate lens condensor. The equipment was built for taking one sample only crossing once the light beam without stopping the carrying wheel. Magnetic stirrers are located on wheel just below cylindric cuvettes; they stop when optical density is recorded. The two fluorescent tubes do not disturb the measurements

our SAFAS recording spectrophotometer (Fig. 2) allowed us to survey the 14 cultures simultaneously, while being shaken by magnetic stirrers and lighted with Sylvania-Grolux fluorescent tubes. The blank medium of reference cultures was prepared as follows: ES solution + 2 g/l bacto peptone + 0.5 g/l beef extract + 0.5 g/l yeast extract; pH: 7.7, sterilization: filtering; *T. striata* grows quicker in this reference medium than in the original ES solution.

Results

Optical density data of *Tetraselmis striata* cultures are plotted in Fig. 3. Obviously, algal growth is enhanced by a slight bacterial population filtrate (OD = 0.065 and 0.072); growth rate is faster and population density higher than in the reference culture. Later (when OD reaches 0.092 and 0.113) the lag phase increases, while the maximal density remains more or less equal. When OD values are higher than 0.137, the *T. striata* cells are killed. It should be noted that inhibition appears at the beginning of synthesis of the green fluorescent pigment of *Pseudomonas aeruginosa*. As far as the growth curves are parallel, we express the development of *T. striata* as follows:

$$\text{Development} = \frac{\text{Maximal density}}{\text{Time needed to reach maximal density}}.$$

Results were expressed as per cent of reference culture growth (Fig. 4). They clearly indicate that *T. striata* is strongly inhibited by *P. aeruginosa*; algal growth is nil, since the bacteria has reached the half way point of its logarithmic phase.

The tremendous inhibitory effect of *Pseudomonas aeruginosa* leads us to ask: are other bacteria poisonous for algae? We attempted to clarify this problem

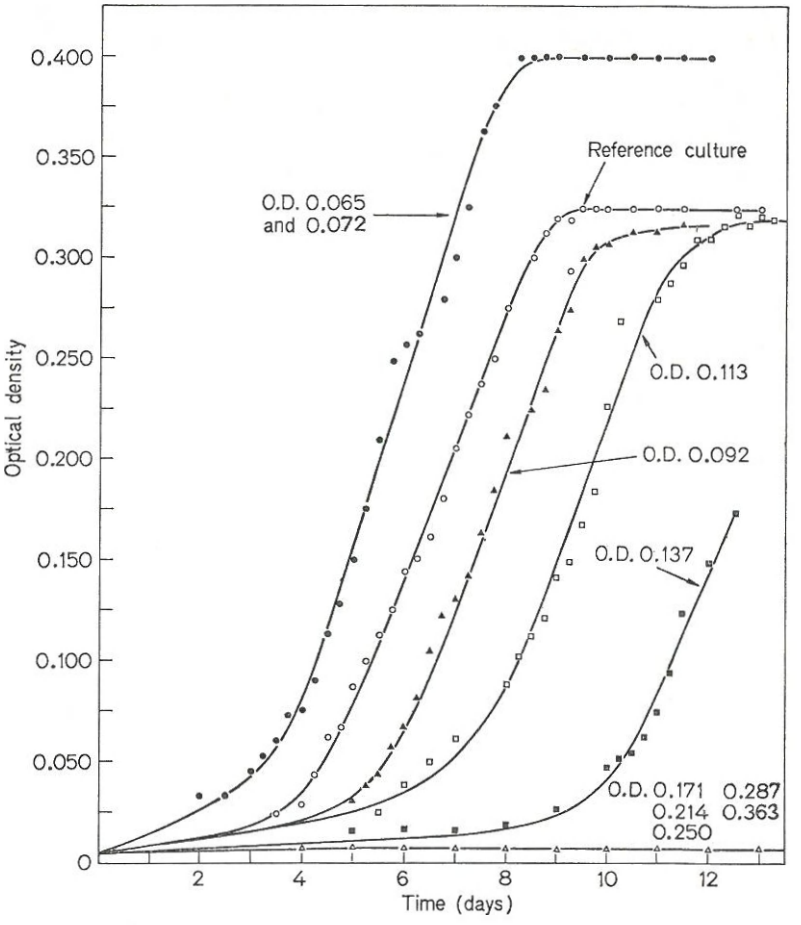

Fig. 3. *Tetraselmis striata*. Growth in ES solutions prepared from *Pseudomonas aeruginosa* cultures, centrifuged at different steps of growth. Algal and bacterial developments are expressed in optical density/cm pathlength of culture. O.D.: optical density

using several bacterial strains of different origin, and bacteria-free cultures of marine algae.

Screening experiments

Methods

The technique described earlier was used, but modified as follows:

(1) Bacteria were grown in a simple medium: Bacto peptone: 1 g; yeast extract: 0.5 g; 80% sea water: 1 l; pH: 7.5—7.8; sterilization: autoclaving (20 mn, 120 °C). Cultures were incubated in the same 40 mm diameter tube until just before they reached the end of logarithmic growth. A previous study had given the average growth curve for each strain used.

(2) ES-filtrate solutions were poured into 18 × 180 mm test tubes, containing 10 ml medium.

(3) After inoculation by bacteria-free cultures, test tubes were incubated at 18 °C, under a continuous light provided by Sylvania-Grolux fluorescent tubes.

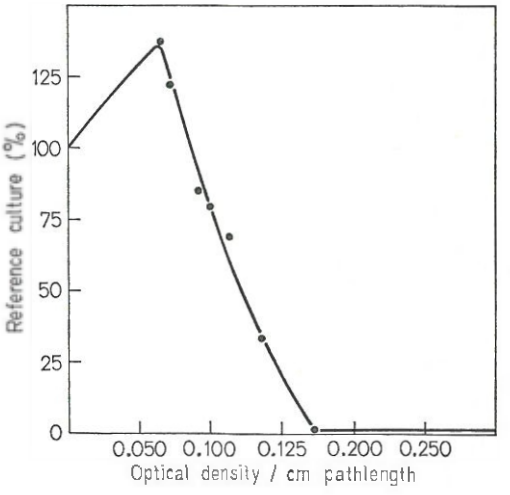

Fig. 4. *Tetraselmis striata* development as a function of optical density of *Pseudomonas aeruginosa* cultures

After a period of at least a week, results were read by direct observation, using a 4° scale as follows: complete inhibition (I) — the experimental tubes are completely clear; inhibition (εI) — there is evidence that test cultures are less deeply coloured than reference culture; no inhibition (0) — test and reference cultures are similar; growth enhancement (GE) of algae. The blank medium was prepared as follows: ES solution + 1 g/l peptone + 0.5 g/l yeast extract; pH: 7.7; sterilization: filtering.

Results

All data of the large-scale survey will be published later, but the primary results, summarized in Table 1, clearly indicate that several bacteria are capable of strong inhibition against algae. *Asterionella japonica* and *Chaetoceros lauderi* appear to be damaged by all the strains tested. Regarding the bacteria, some strains badly damage almost all the algae, especially *Pseudomonas aeruginosa*, *P. marinoglutinosa* and strain reference 00.30.

Discussion and conclusion

The *in vitro* experiments reveal an obvious inhibition of several marine algae by some bacteria. *Pseudomonas aeruginosa* and related strains are not marine organisms, but they can be transported to the sea, especially in polluted areas.

In so far as there is no quantitative similarity between *in situ* and culture densities of bacterial populations, bacterial poisons may only be of some importance when concentrated, as, for example, in narrow areas of waters rich in suspended or dissolved organic matter.

Table 1. *List of marine algae tested and number of inhibiting bacteria strains. I: complete inhibition; εI: inhibition*

Algal species	Number of bacteria tested	Number of (I)	Number of (εI)
Chlamydomonas magnusii	23	6	1
Prasinocladus marinus	13	2	4
Tetraselmis striata	32	6	3
Amphora sp.	20	11	
Asterionella japonica	6	6	
Chaetoceros lauderi	5	5	
Hemiaulus sinensis	19	7	1
Nitzschia acicularis	8	2	6
Phaeodactylum tricornutum	16	2	1
Thalassiosira rotula	1	1	
Stichochrysis immobilis	20	7	1
Heterothrix sp.	20	2	3
Monallantus salina	20	4	

There is, however, no indication at present that bacterial toxins are capable of playing a role in the algae-bacteria relationships in the sea as a whole.

Summary

1. *In vitro* experiments indicate that the pigmented poison of the bacterium *Pseudomonas aeruginosa* is a strong growth inhibitor of the alga *Tetraselmis striata*.

2. Several bacteria strains from different origins are recognized to have the same toxicity against various marine algae.

3. Taking into account the very great differences between *in vitro* experiments and *in situ* environmental conditions, it is not at present possible to state that bacterial poisons really play a role in the algae-bacteria relationships in the sea.

Acknowledgements. Our thanks are due to Dr. P. Bourrelly (Museum, Paris), Dr. M. J. Chretiennot (Station marine d'Endoume) and Professor P. Gayral (University of Caen) for determining several micro-algae; to Dr. M. Aubert (C.E.R.B.O.M., Nice) and Dr. A. Bianchi (Station marine d'Endoume) for providing several strains tested.

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Date of final manuscript acceptance: September 9, 1971. Communicated by J. M. PERES, Marseille