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Study of bacteria associated with marine algae in culture

II. Action of antibiotic substances

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Abstract

Inhibitory action of some usual antibiotic substances have been tested on 25 species of bacteria isolated from marine algae cultures. Penicillin, kanamycin, neomycin and streptomycin appear to be the most convenient antibiotics for eliminating or inhibiting polluting bacteria populations.

Introduction

Washing and plating technics are much more successful in obtaining bacteria-free marine algae cultures, when the bacteria population is previously decreased with low amounts of antibiotics. The present

Table 1. *Minimum inhibitory concentrations (µg/ml) of antibiotics*

Genera and species	Aureo-mycin	Terra-mycin	Penicillin	Kana-mycin	Neomy-cin	Strepto-mycin	Dihydro-strepto-mycin	Chloram-phenicol	Poly-myxin B
Pseudomonas									
P. aestumarina	300	200	4000	200	20	>10000	> 5000	100	> 900
P. cruciviae	60	50	1	30	30	30	25	2	4
P. marinoglutinosa	400	1000	250	400	100	400	1000	150	15
P. (marinoglutinosa)	300	1500	1500	40	40	30	20	25	10
P. riboflavina	50	20	50	20	20	20	75	25	20
P. stereotropis	80	175	0.25	200	50	150	25	2	25
P. sp. 1	200	700	2000	50	60	30	25	15	50
P. sp. 2	200	750	750	70	50	200	200	7.5	10
P. sp. 3	40	100	12	200	30	100	>10000	1.5	> 900
Vibrio									
V. algosus	400	150	1750	400	200	300	200	10	5
V. phytoplanktis	300	175	15	400	175	80	150	1.5	10
Agarbacterium									
A. mesentericus	200	1500	3000	100	30	20	60	5	50
Xanthomonas									
X. sp. 1	100	100	30	100	1500	>10000	> 5000	1	500
Achromobacter									
A. parvulus	80	150	1	200	125	>10000	200	2	500
A. stationis	15	50	0.25	7	10	15	15	1.5	50
A. stenohalis	50	20	50	60	15	5	10	1.5	2
A. sp. 1	200	750	750	50	30	40	25	2	7.5
Flavobacterium									
F. aquatile	200	125	50	>5000	>1000	>10000	> 5000	30	>1000
F. lutescens	300	75	100	>5000	>2100	10000	9500	75	>1000
F. peregrinum	30	100	2	30	10	7.5	5	5	150
F. sp. 1	30	40	0.05	80	30	>10000	125	0.75	2
F. sp. 2	300	100	750	>5000	>1000	>10000	> 5000	100	>1000
F. sp. 3	400	150	400	60	>2100	10000	9500	200	600
Micrococcus									
M. sp. 1	50	250	15	80	400	25	100	2	150
Staphylococcus									
S. aureus	40	150	7	9	30	20	20	5	400
S. aureus peni. (—)[a]	20		150						
S. aureus peni. (+)			>1000						

[a] *S. aureus* penicillin (—) and *S. aureus* penicillin (+) are collection strains from the "Faculté de Pharmacie de Marseille", France.

study is concerned with minimum inhibitory concentrations of some of the usual antibiotics. Their effects were tested on 25 bacteria species.

Material and methods

Bacteria strains were isolated from marine algae cultures as previously described (Berland et al., 1969). ZoBell's (1941) medium number 2216 was used; it was autoclaved after adjusting the pH to 8. Antibiotic solutions were added (1 ml per 9 ml medium) through a sterilizing Swinnex Millipore filter holder, containing one GS 0.22 micron Millipore filter. Inocula cells were harvested from a 24 to 48 h plate culture, then put in a sterile solution and gently Vortex-shaked. About 250×10^3 cells were inoculated in order to reach the bacteria density most frequently observed in our polluted algae cultures. Experiments were carried out in triplicata test tubes, incubated for a week at room temperature. In experiments yielding uncertain results, the tests were repeated two or three times.

Results and conclusions

Among representatives of one and the same genus, minimum inhibitory concentrations (Table 1) do not attain the same magnitude for every species, except neomycin and polymixin B with *Pseudomonas*. Some bacteria strains appear to be resistant to all tested substances, especially *Pseudomonas aestumarina* and *Flavobacterium* sp. 2. As regards streptomycin and dihydrostreptomycin, which are well known to produce resistant mutagens, some of the strains studied (chiefly the Flavobacteria) are not injured until high concentrations are reached. In contrast, we found other species susceptible to very low concentrations, e.g. *Flavobacterium peregrinum*.

Table 2. *List of antibiotic substances inhibiting some bacteria strains in low concentrations*

Antibiotics	Species inhibited	Concentrations (µg/ml)
Bacitracin	*Pseudomonas cruciviae*	100
	Pseudomonas stereotropis	100
	Pseudomonas sp. 3	100
	Xanthomonas sp. 1	100
	Achromobacter parvulus	100
	Achromobacter stenohalis	100
	Flavobacterium peregrinum	5
	Flavobacterium sp. 1	100
	Micrococcus sp. 1	5
Spiramycin	*Achromobacter parvulus*	3
	Achromobacter stationis	3
	Flavobacterium peregrinum	3
	Flavobacterium sp. 2	9
	Micrococcus sp. 1	3
Vancomycin	*Xanthomonas* sp. 1	12
	Achromobacter parvulus	3
	Achromobacter stationis	9
	Flavobacterium peregrinum	3
	Micrococcus sp. 1	9
	Staphylococcus aureus	9
Sulfamerazin	*Flavobacterium* sp. 2	15
Triple Sulfa	*Flavobacterium* sp. 2	15

Four substances are capable of strongly inhibiting bacteria; aureomycin, terramycin, and especially polymixin B and chloramphenicol, which severely harm the bacteria cells at very low concentrations; unfortunately, the same concentrations also critically damage the algae (Berland and Maestrini, 1969) and hence are not suitable for our purpose.

Several other antibiotics have been tested, most of them are similar to chloramphenicol; the results have, nevertheless, been noted in Table 2.

Among the antibiotics tested, penicillin, kanamycin, neomycin and streptomycin appear to be the most convenient ones for elimination or inhibition of the bacteria population of marine algae cultures.

Summary

1. In order to assess the eliminatory or inhibitory effects of antibiotic substances on bacteria populations often found to pollute marine algae cultures, the action of some antibiotics have been tested.

2. Different bacteria species belonging to the same genera do not exhibit the same degrees of resistance to the antibiotics employed.

3. Several antibiotic substances are strong bacterial inhibitors, but unfortunately also critically injure the cultivated marine algae. The most convenient antibiotic substances appear to be penicillin, kanamycin, neomycin and streptomycin.

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