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Effects of technetium on marine micro-organisms *

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Summary

Eleven bacterial species have been isolated from the upper layer of intertidal sediments collected along the Belgian coast (Coxyde). Three of them (n° 1, 4 and 11) have been chosen for their halophilous character. One species has been identified as *Flavobacterium halmephilum,* the other two are being investigated. Effects of technetium (^{99}Tc) have been studied on a mixed bacterial population isolated from sediments, as well as on the three halophilic species.

At the concentrations utilized in this work (up to 100 µg ml^{-1}), ^{99}Tc has no evident effects on bacterial growth. Uptake of technetium (^{99}Tc and/or ^{95m}Tc) has been investigated in the mixed bacterial population, in the three halophilic bacteria (including *Flavobacterium halmephilum*) and in the benthic ciliate *Uronema marinum.* It has been found that technetium is taken up by all these micro-organisms. However, the transfer factor (TF) in bacteria may vary considerably (from 0.5 to 200), but the cause of this variability is not known and deserves further study.

The ciliate *Uronema marinum,* which feeds on living marine bacteria, was found to take up ^{95m}Tc added to the culture medium. However, the TF in this ciliate is rather low (from 1.4 to 5.5). Because it feeds on bacteria, *Uronema marinum* is supposed to take up technetium from water (direct contamination) as well as from contaminated bacteria (indirect contamination). Experiments with ^{95m}Tc-labeled bacterial cells might be useful, as they could indicate which form of contamination (direct or indirect) is prevailing.

Key-words : marine bacteria, ciliates, halophilism, technetium.

Résumé

Onze souches bactériennes ont été isolées de la couche superficielle de sédiments prélevés le long de la côte belge (Coxyde). Trois souches (n° 1, 4 et 11) ont été choisies pour leur caractère halophile. Une espèce a été identifiée comme *Flavobacterium halmephilum* et les deux autres bactéries sont à l'étude. Les effets du technétium (^{99}Tc) ont été étudiés sur ces trois souches de même que sur une population bactérienne mixte.

Aux concentrations utilisées dans ce travail (jusqu'à 100 µg ml^{-1}), le ^{99}Tc n'a pas d'effets évidents sur la croissance bactérienne. La fixation du technétium (^{99}Tc et/ou ^{95m}Tc) a été étudiée dans les trois souches de bactéries dont *Flavobacterium halmephilum,* dans une population bactérienne mixte et dans le cilié benthique *Uronema marinum.* Il a été observé que le technétium est fixé par tous ces micro-organismes. Cependant, le facteur de transfert (FT) dans les bactéries peut varier fortement (de 0,5 à 200). La cause de cette variation n'est pas connue et nécessite une étude plus approfondie.

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Le cilié *Uronema marinum,* qui se nourrit de bactéries marines vivantes, est capable de fixer le ^{95m}Tc ajouté au milieu de culture. Toutefois, le FT dans cet organisme est plutôt faible (de 1,4 à 5,5). On peut supposer que la fixation du technétium dans *Uronema marinum* a lieu directement, à partir de l'eau, et indirectement, par le biais des bactéries contaminées. Des expériences avec des bactéries marquées au ^{95m}Tc pourraient révéler quelle forme de contamination (directe ou indirecte) prévaut dans l'écosystème côtier.

Mots-clefs : bactéries marines, ciliés, halophilisme, technétium.

Introduction

Among the 21 isotopes of technetium, which is a fission product of uranium, thorium and plutonium (WILDUNG *et al.*, 1979), ^{99}Tc (half-life of 2.1×10^5 years) is considered to be a potential pollutant for the environment. The γ-emitting isotope ^{95m}Tc (half-life of 61 days) is advantageously used as tracer of ^{99}Tc, especially *in vivo.*

Within a joint research program on technetium behaviour in the marine environment, we have investigated the uptake of this radionuclide in micro-organisms. The first part of our work deals with the behaviour of certain marine bacteria in the presence of Tc; the second, with the reaction of a marine ciliate, *Uronema marinum,* to Tc and to bacteria labeled with this radionuclide.

1. Behaviour of marine bacteria in the presence of technetium

1.1. *Numbering of marine bacteria*

After adding to Petri dishes containing gelose (i.e. 2.4 % Tryptone Glucose Extract, Agar, Difco) aliquots of interstitial water extracted aseptically from the Belgian coast sand (collected at Coxyde, in the surface sand of the intertidal zone), we have numbered the bacteria which were able to grow at 20° C. After 10 days of incubation, we detected 11,500 bacteria per ml of interstitial water (average of 10 dilutions, with 2 Petri dishes per dilution).

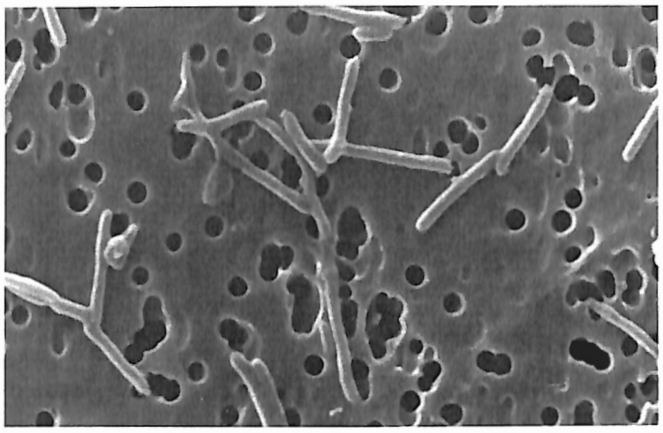

Fig. 1. Flavobacterium halmephilum. *Scanning electron micrograph of cells grown in the laboratory and collected on a 0.45 µm Nucleopore filter. Scale = 1 µm.*

1.2. *Isolation of marine bacteria*

One of the Petri dishes contained 11 different colonies, distinctly separated one from the other. We have isolated, purified and numbered them from 1 to 11.

1.3. *Description of Flavobacterium halmephilum*

Among the 11 isolated strains we have retained 3, because of their halophilic character. They were identified according to BUCHANAN and GIBBONS (1975). Strain nr 1 corresponds to *Flavobacterium halmephilum* (ELAZARI-VOLCANI) (fig. 1) : small, non motile rods, strictly aerobic; yellow pigmentation; using neither glucose, nor lactose, nor most of the glucides; no catalase; strictly halophilic; no growth at 37° C; maximum growth after 5 days, on bacto-peptone, at 20° C. The other bacteria strains shall be described later on (work in progress).

1.4. *Effects of ^{99}Tc on the growth of a mixed bacterial population*

In order to measure the resistance to ^{99}Tc, we have cultivated and numbered the total of bacteria in the interstitial water, in the presence of gelose containing respectively 1, 10 and 100 µg ml^{-1} of ^{99}Tc (Amersham). The results obtained are reported in Table 1.
It therefore appears that ^{99}Tc has no detrimental effect on the development of bacteria. On the other hand, a brown pigmentation appeared on the colonies, the next day already for the cultures at 100 µg ml^{-1}, and later on for those at 10 µg ml^{-1}. However, a microscope examination of these bacteria revealed no obvious difference in morphology with respect to those grown in the absence of technetium.

1.5. *Uptake of ^{99}Tc in strains nr 1, 4 and 11*

We have experimented with two different technetium concentrations, in order to find out whether or not the transfer factor decreased with the increase of Tc in the medium, as it has been noted for some plants and bacteria (VANDECASTEELE, 1981). Culture tubes with 10 ml of bacto-peptone medium containing respectively 1 and 10 µg ml^{-1} of ^{99}Tc were prepared and inoculated with one loop of a 24-hour culture of each strain. After 6 days, the solutions were filtered through a 0.45 µm Millipore membrane. The culture tube was rinsed with a 10 ml of non contaminated fresh medium, which was then passed through the above mentioned filter. A control solution underwent the same treatment, thus permitting to determine the percentage of ^{99}Tc retained by the membrane. In order to reduce the coloration of the sample, 4 ml of sea-water were added to one ml of each filtrate. Moreover, 2 drops of 40 % formol were added too, in order to prevent bacterial development. After a final addition of 5 ml Instagel (Packard), ^{99}Tc was measured by liquid scintillation (Spectrophotometer Packard Tri Carb, model 2450).
By comparing the radioactivity of the control solution with that of the filtrate, and after correcting the quenching by using an internal standard, the percentage of ^{99}Tc taken up by a given number of bacteria was determined.
The average weight of the bacteria of each strain has been calculated. A 6-day culture (500 ml) was centrifuged for 30 min. at 12,000 rpm. The pellet was then rinsed, centrifuged and weighed immediately, and again after drying. The numbering was made on 1/10 ml of the same culture. If the total weight, the number of bacteria and the volume of the culture are known, it is possible to calculate the average weight of one bacterium, and thereafter the transfer factor. The results obtained with 1 and 10 µg ml^{-1} of ^{99}Tc are reported in Table 2 (upper part).

1.6. *Concentration of ^{99}Tc in a mixed bacterial population*

It has been well-known for a long time that environmental bacteria numbering may strongly depend upon the type of technique used. Researchers are well aware of the fact that enrichment media may be very selective. For instance, ROMANENKO's (1979) experiments to this purpose are quite conclusive.
In order to obtain the sediment microflora as natural as possible, interstitial water samples were placed in seawater containing 300 mg l^{-1} peptone. Under our experimental conditions, this slight addition of nutrient appeared to be necessary to stimulate bacterial growth. This type of

Table 1
Effects of ^{99}Tc on the growth of marine bacteria.

Concentration of ^{99}Tc (µg ml^{-1})	Number of bacteria ml^{-1}
0	50,000
1	87,000
10	62,000
100	62,000

Table 2
Uptake of ^{99}Tc by marine bacteria.

Bacterial population	Culture medium in sea-water	Technetium concentration (µg ml^{-1})	Number of bacteria ml^{-1}	Transfer factor (1)
strain 1	peptone 10 g l^{-1}	1	80.10^7	2
strain 4	peptone 10 g l^{-1}	1	17.10^7	20
strain 11	peptone 10 g l^{-1}	1	44.10^7	15
mixed	peptone 10 g l^{-1}	10	2.10^7	53
mixed	peptone 0,3 g l^{-1}	10	$3{,}8.10^6$	212
mixed	peptone 0,3 g l^{-1}	1	$2{,}1.10^7$	0,5

(1) $TF = \frac{\text{cpm g}^{-1}\text{ bacteria}}{\text{cpm ml}^{-1}\text{ medium}}$

culture has been done simultaneously in parallel with a traditional culture on bacto-peptone at 10 g l^{-1}. Both types of media have been inoculated with 10 µl of interstitial water, to which either 1 or 0 µg ml^{-1} of ^{99}Tc were added. After 6 days, the amount of ^{99}Tc was measured in the filtrates of the cultures, as previously described. The results obtained are shown in Table 2 (lower part).

1.7. *Concentration of ^{95m}Tc in Flavobacterium halmephilum*

Under our culture conditions (21 ± 1° C; alternation of 12 h light (800 lux) and 12 h darkness), *Flavobacterium* is capable of taking up ^{95m}Tc. However, the transfer factor found in two different experiments remains rather low (Table 3). The quantity of ^{95m}Tc taken up per g of bacteria is nevertheless sufficient to allow transfer experiments along the trophic chain.

2. Behaviour of *Uronema marinum* in the presence of technetium

2.1. *Description*

Uronema marinum (DUJARDIN, 1841) (fig. 2) : Marine benthic ciliate; holotriche hymenostome, described by KAHL

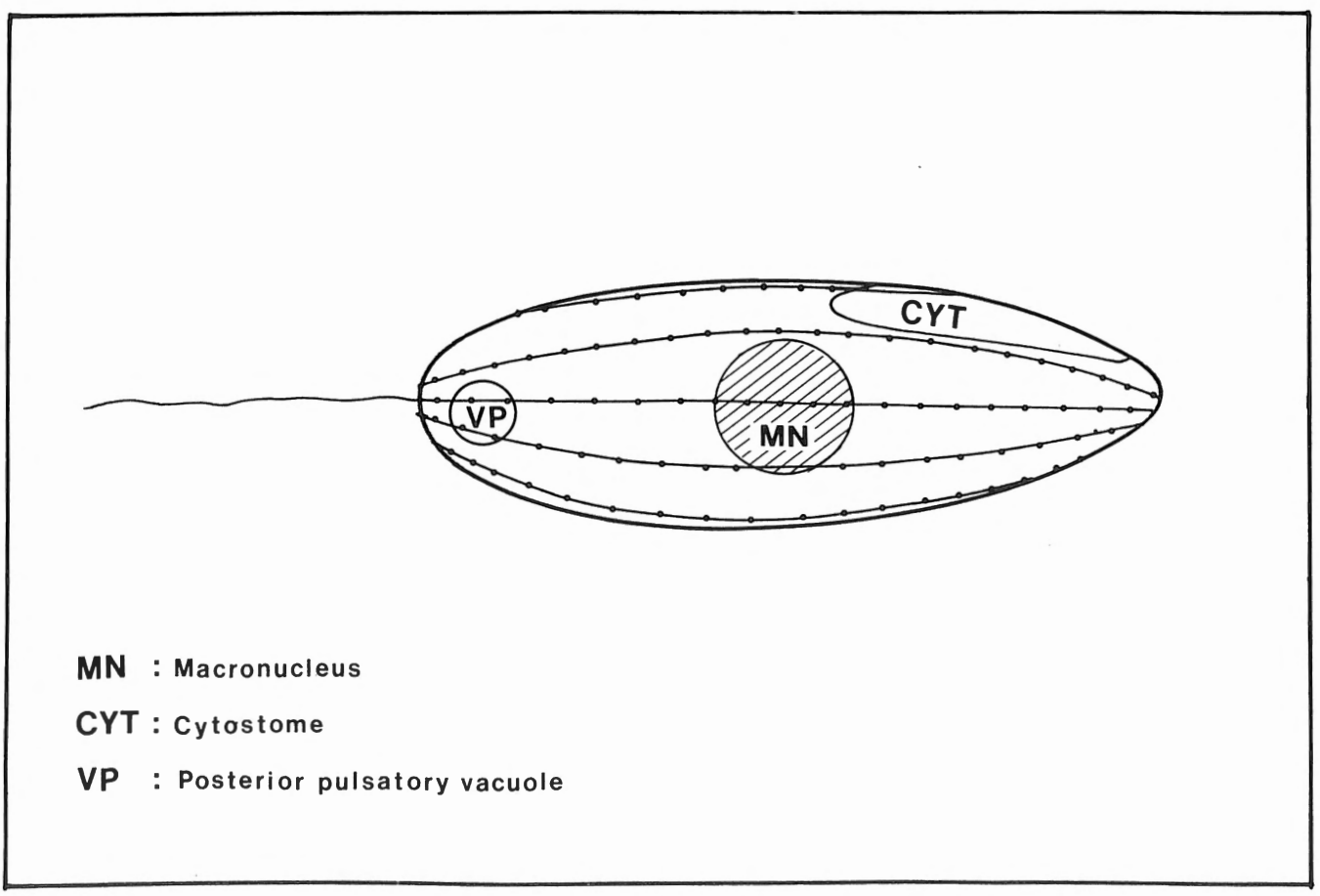

Fig. 2. Uronema marinum. *Line drawing showing the cellular morphology of this marine benthic ciliate, which has a uniform ciliature but one single long caudal cilium. The average length of the body is 50 µm.*

(1930); reproduction potential studied by FENCHEL (1968); reported in the sand of the Belgian coast by CHARDEZ (1972); found in the coastal sand, at 2 m depth, by DARTEVELLE (1975). Swimming form, circulating between grains of sand and ingesting bacteria by the motion of its buccal membranelles; oval body, of 50 µm average length, 20 µm average breadth, and consequently, 0.1 mm^3 average volume; uniform ciliature, except for one caudal cilium that is longer; posterior pulsatory vacuole; spheric macronucleus; circular cysts of 15 to 17 µm size.

Table 3
Uptake of ^{95m}Tc by Flavobacterium halmephilum.

Experiment	Radioactivity		
	Culture medium nCi ml^{-1}	*F. halmephilum* nCi g^{-1}	Transfer factor (TF)
A (a 4-day old culture)	35	83.6	2.4
B (a 7-day old culture)	54	123	2.3

2.2. *Culture*

2.2.1. Culture in the presence of a mixed population of marine bacteria

One *Uronema,* rinsed 10 times with sterile sea-water, was placed in 100 or 200 ml of sea-water, filtered through PRAT-DUMAS nr 4 filter paper. Bacto-peptone Difco (0.25 mg ml^{-1}) was added, and the incubation took place at laboratory temperature (± 20 ° C) in the presence of a mixed bacterial population. The *Uronema* rapidly multiplied, at the expense of different marine bacteria which develop in this peptone-enriched medium. Under our experimental conditions, the Protozoa culture reached its maximum growth at the end of a 5-day period, after which it slightly decreased. It is, however, possible to maintain a growing culture by adding empirically a small quantity of peptone when the number of bacteria becomes too low. A better control of the culture, for both *Uronema* and the bacteria, would be possible by using chemostats (CURDS and COCKBURN, 1971). In order to measure the growth rate, the Protozoa present in an aliquot of the culture were regularly counted.

2.2.2. Culture in the presence of a pure strain of bacteria

The method used is the same as the one previously described, except that the medium was sterilized and inoculated with a pure strain of marine bacteria. The purity thereof was periodically checked by isolation on gelose. Since the monoxenic culture is perfectly well tolerated by *U. marinum,* feeding with ^{95m}Tc-labeled bacteria could be undertaken.

2.3. *Method used for counting Uronema marinum*

This counting cannot be done by measuring the optical density, since the density of the bacteria population on which the Protozoa feed varies in the course of time, thus modifying the turbidity of the medium. A suitable method was therefore applied consisting of the following stages :

a) 20 µl of the culture were placed upon a 0.45 µm pore size Millipore grid marked filter;
b) one drop of DA FANO fixative prepared with sea-water (LANGERON, 1949) was added;
c) after 3 min. fixation, the solution was filtered through the membrane;
d) the membrane was immediately rinsed with one drop of sterile sea-water which was afterwards aspirated;
e) one drop of carmine alun solution of GRENACHER (LANGERON, 1949) was added and left to react for a few seconds before it was aspirated;
f) one drop of immersion oil was placed on the membrane. In order to eliminate all water, the membrane was placed during one night in a stove, at 37° C;
g) the Protozoa were then counted.

2.4. *Remarks on the culture of Uronema marinum*

The following remarks on the culture of *U. marinum* are of practical interest :

a) Attemps to feed cultures of *U. marinum* with different strains of killed marine bacteria, suspended in sea-water, were unsuccessful;
b) *U. marinum* resists to a mixture of penicilline (100 U ml^{-1}) and streptomycine (50 µg ml^{-1}), concentrations which are normally used for cell-cultures. However, since many marine bacteria resist to these antibiotics, the method is not suitable for obtaining axenic cultures of *Uronema*;
c) Under our experimental conditions, the concentration of bacterial food was not controlled. This concentration has, of course, an influence on the growth constant "K", as it has been shown by FENCHEL (1968). This author reported that *U. marinum* can multiply at 0° C, but that it grows optimally at 20° C.

2.5. *Growth curve*

The growth curve is obtained by counting the Protozoa in aliquots of culture medium at regular intervals. By using the log of the number of Protozoa, a linear plot is obtained (fig. 3) and an adjustment test is made on the linear regression. Under our experimental conditions, we obtained a regression factor (r^2) of 0.91 (which indicates an excellent curve adjustment) and an 8-hour generation period. The growth rate would of course be higher in a continuous culture. FENCHEL (1968) succeeded in obtaining a minimum generation period of 2.5 h.

Table 4
Uptake of ^{95m}Tc by Uronema marinum *and by a mixed population of marine bacteria.*

Experiment	Radioactivity and transfer factor (TF)				
	Culture medium nCi ml^{-1}	*Uronema* nCi g^{-1}	T.F.	Bacteria nCi g^{-1}	T.F.
A (a 4-day incubation)	20	111	5.5	197	9.8
B (a 4-day incubation)	33	47	1.4	71	2.1

2.6. *Uptake of ^{95m}Tc by Uronema marinum*

Since *U. marinum* does not grow in the absence of living bacteria, mixed cultures (bacteria and *Uronema*) have been contaminated with ^{95m}Tc. At the end of the incubation, *Uronema* and bacteria were harvested by centrifugation at respectively 1000 and 9000 rpm, during 10 min. It is quite probable that the *Protozoa* thus separated were contaminated by bacteria upon which they fed. The results obtained in two different experiments are reported in Table 4.

3. Discussion

Investigation on the uptake, distribution and biological effects of radionuclides in sea-organisms are of general interest to assess the impact of artificial radionuclide releases on life in the marine environment as well as on contamination of food chains leading to man.
Marine micro-organisms might play an important role in the recycling of radioactive materials and in their transfer to higher trophic levels. However, information on the interaction of marine microbiota with artificial radionuclides remains rather scarce (see literature in Strack *et al.*, 1980 and in Arapis *et al.*, 1987).
This paper deals with the impact of technetium on marine bacteria and on the benthic ciliate *Uronema marinum*, and is intended to be a contribution to a better understanding of the behaviour of this artificial radionuclide in the biological portion of the biogeochemical cycle.
Results reported here show that ^{99}Tc, at the concentrations utilized in our experiments (up to 100 μg ml^{-1}), has no significant effect on bacterial growth. However, since this radionuclide is taken up by the bacterial cell — the transfer factor varying from 0.5 to about 200 — it might produce some other kind of effects (for example physiological and biochemical) not investigated in this study. Further work is needed to reveal possible physiological and/or biochemical effects of technetium on marine bacteria. The reason why the transfer factor of ^{99}Tc in bacteria varies considerably remains unknown. More insight into the physical and chemical factors affecting the uptake of technetium in marine bacteria should first be gained in order to elucidate this phenomenon.
The sensitivity of several micro-organisms to ^{99}Tc has been investigated by Gearing *et al.* (1975). They reported that growth of the non sulfur purple bacterium *Rhodo-*

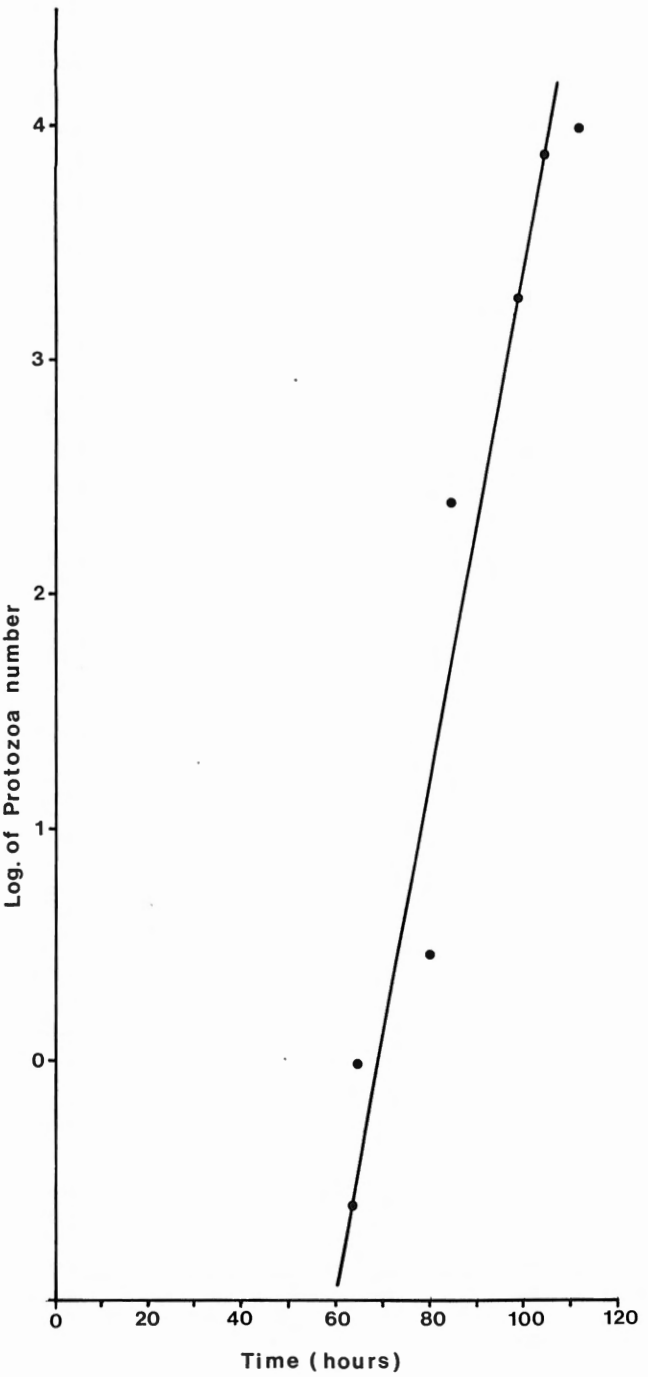

Fig. 3. *Growth of* Uronema marinum *under laboratory conditions. This linear plot, obtained by using the log of Protozoa number versus time, gives a generation period of 8 h. The regression factor r^2 is 0.91. The straight line equation is : $Y = 0.11 \times - 7.73$.*

spirillum rubrum was strongly inhibited already at 1 µg ^{99}Tc ml^{-1}. However, the other micro-organisms investigated, were much less sensitive. The above results as well as our own indicate that the response of marine bacteria to technetium (pertechnetate) may vary considerably from one species to another.

The marine benthic ciliate *Uronema marinum* is capable of accumulating ^{95m}Tc present in the external medium, although only to a limited extent (TF from 1.4 to 5.5). Since *Uronema* feeds on living bacteria, technetium uptake is supposed to occur from water (direct contamination), as well as from contaminated bacteria (indirect contamination). Feeding experiments with ^{95m}Tc-labeled bacterial cells may be useful, to find out which form of contamination (direct or indirect) prevails.

The results reported in this paper raise the following important questions :

a) Do sedimentary marine bacteria interact with radionuclides as the free-living bacteria in the water column or not ?
b) Are marine bacteria to be regarded as remineralizers of radioactive materials ?
c) To which extent do bacteria (contaminated or not by radionuclides) contribute to the nutrition of marine animals ?
d) Which role do the Ciliates like *Uronema marinum* play in the uptake, distribution and transfer of radionuclides in the marine ecosystem ?

Most studies on the ecological role of free-living bacteria in the water-column and the sedimentary ones are rather recent (see literature in AZAM *et al.*, 1983 and in FALLON *et al.*, 1983). It is known that pelagic bacteria utilize the dissolved organic matter (DOM), originated mainly from phytoplankton species, and to a smaller extent from marine plants (mainly debris) and animals (SEPERS, 1977; LANCELOT, 1979; LARSSON and HAGSTRÖM, 1979; SCHLEYER, 1980; LINLEY *et al.*, 1982; WOLTER, 1982; TENSEN, 1983). Non radioactive and radioactive DOM (contaminated with ^{3}H, ^{14}C or other released radionuclides) are probably utilized by pelagic bacteria with the same efficiency. Even if a sufficient amount of DOM is available, the density of bacteria in the water column is kept under control by heterotrophic flagellates, which are in turn preyed upon by microzooplankton (see AZAM *et al.*, 1983). A similar prey-predator relationship probably exists for sedimentary bacteria. The latter are not expected to differ very much from pelagic bacteria in their ability to take up DOM and the released radionuclides. However, since the small size (average of 0.09 µ^3 cell^{-1} according to FERGUSON and RUBLEE, 1976) and large surface-to-volume ratio of bacterial cells permit absorption of nutrients at very low concentrations (AZAM *et al.*, 1983), sedimentary bacteria are probably capable of incorporating very efficiently radionuclides diffused from contaminated sediments on which they live, even if the diffusion rate is particularly low. Culture of sedimentary bacteria on sediments labeled with technetium (or with other radionuclides) would enable us to answer this question.

Marine bacteria have been regarded as remineralizers, that is, as organisms capable of converting organic to inorganic matter and of recycling nutrients for primary producers such as phytoplankters. However, recent evidence obtained by AZAM *et al.* (1983), suggests that remineralization in the sea is mainly assured by heterotrophic flagellates and microplankton. The latter, if the above view is correct, would thus recycle, at least in part, the radioactive material taken up by pelagic or sedimentary bacteria.

It appears already from early studies (ZOBELL and LANDON, 1937; ZOBELL and FELTHAM, 1938), that marine bacteria may play an important role in the nutrition of marine animals. Mussels (*Mytilus californianus*), sand-crabs (*Emerita analoga*) and worms (*Dendrostroma zostericola* and *Urechis caupo*) were found to eat bacteria and to derive nourishment therefrom (ZOBELL and FELTHAM, 1983). Later work has confirmed that marine bacteria are used as nourishment by several animals (such as sponges, bivalves and worms), (see literature in AZAM *et al.*, 1983). Recent studies on the common mussel (*Mytilus edulis*) and on other marine bivalves have revealed the existence of lysozyme in their digestive system (MCHENERY *et al.*, 1979; MCHENERY and BIRKBECK, 1982; BIRKBECK and MCHENERY, 1982). The enzyme would mainly function in the degradation of bacteria. However, lysozyme resistent bacteria, as *Micrococcus roseus* and *Staphylococcus aureus*, cleared by the mussel from the medium, are rejected intact (BIRKBECK and MCHENERY, 1982). Thus, not all the bacteria ingested may be used by the animal. More information on the sensitivity of the different species of marine bacteria to lysozyme would be of great help to evaluate the extent to which they contribute to the nutrition of bivalves and probably of other marine animals as well. However, most of marine bacteria are not used by large animals, but by small heterotrophic flagellates (see AZAM *et al.*, 1983). Possibly, radionuclides taken up by sedimentary or free-living bacteria, would thus be transferred to higher trophic levels by the *Protozoa*, which are in turn eaten by microzooplankters. The benthic ciliate *Uronema marinum*, used in this work, may be a valuable experimental subject for a study of the transfer of technetum (and other radionuclides) in the marine food chain.

A more accurate estimation of the production rate of different types of micro-organisms (bacteria, flagellates, phytoplankters, etc.) in marine ecosystems, would be most useful for the evaluation of the role they play in the recycling and transfer of artificial radioactivity. The techniques recently developed for estimating production rates of marine bacteria (JANNASCH and WIRSEN, 1980; AZAM *et al.*, 1983; FALLON *et al.*, 1983) might be successfully applied to other types of marine micro-organisms also.

4. Conclusion

Halophilic marine bacteria, isolated from sediments, and the ciliate *Uronema marinum*, are able to take up ^{99}Tc and/or ^{95m}Tc. Though the transfer factors of technetium

in bacteria and the ciliate are not high, they are nevertheless sufficient for following the transfer of this important radionuclide in the marine food chain. Our results suggest that micro-organisms may play a significant role in transferring released radionuclides to higher trophic levels.

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References

Amano, M., Hara, S. & Taga, N., 1982. Utilization of dissolved amino acids in seewater by marine bacteria. *Marine Biology,* 68 : 31-36.

Arapis, G., Bossus, A., Nuyts, G., Bonotto, S., olard, J., Mathot, P. & Cogneau, M., 1987. Radioactive contamination of the marine environment : uptake and distribution of tritium and technetium in *Acetabularia* and other marine algae. In : Capuzzo, J.M. and Kester, D.R. (Editors), Oceanic Processes in Marine Pollution, Vol. 1, Biological Processes and Wastes in the Ocean. Robert E. Krieger Publ. Co., Malabar, Florida, pp. 133-144.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. & Thingstad, F., 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series,* 10 : 257-263.

Birkbeck, T.H. & McHenery, J.G., 1982. Degradation of bacteria by *Mytilus edulis*. *Marine Biology,* 72 : 7-15.

Buchanan, R.E. & Gibbons, N.E., 1975. Bergey's manual of determinative bacteriology. William and Wilkings Baltimore, pp. 1268.

Chardez, D., 1972. Etudes sur les protozoaires psammophiles littoraux, pp. 13. Diffusion restreinte. Institut Agronomique de Gembloux.

Curds, C. & Cockburn, A., 1971. Continuous monoxenic culture of *Tetrahymena pyriformis*. *The Journal of General Microbiology,* 66 : 95-108.

Dartevelle, Z., 1975. Approche d'une écologie microbienne de la zone littorale belge. Thèse doctorat. Université Catholique de Louvain, pp. 319.

Fallon, R.D., Newell, S.Y. & Hopkinsons, C.S., 1983. Bacterial production in marine sediments : will all specific measures agree with whole-system metabolism. *Marine Ecology Progress Series,* 11 : 119-127.

Fenchel, T., 1968. The ecology of marine microbenthos. III. The reproductive potential of Ciliates. *Ophelia,* 5 : 123-136.

Ferguson, R.L. & Rublee, P., 1976. Contribution of bacteria to standing crop of coastal plankton. *Limnology and Oceanography,* 21 : 141-145.

Gearing, P., Van Baalen, C. & Parker, P.L., 1975. Biochemical effects of technetium-99 pertechnetate on micro-organisms. *Plant Physiology,* 55 : 240-246.

Hussenot, J. & Laurent, M., 1973. Evaluation de la production bactérienne d'un étang de Sologne. *Annales d'Hydrobiologie,* 4: 169-181.

Jannasch, H.W. & Wirsen, C.O., 1980. Studies on the microbial turnover of organic substrates in deep sea sediments. In : Biogéochimie de la matière organique à l'interface eau-sédiment marin. Colloques Internationaux du C.N.R.S., nr 293, pp. 285-290.

Jensen, Z.M., 1983. Phytoplankton release of extracellular organic carbon, molecular weight composition, and bacterial assimilation. *Marine Ecology Progress Series,* 11 : 39-48.

Kahl, A., 1930. Urtiere oder Protozoa. I. Wimpertiere oder Ciliata (Infusoria). Die Tierwelt Deutschlands, Jena, Verlag von Fischer, pp. 886.

Lancelot, C., 1979. Gross excretion rates of natural marine phytoplankton and heterotrophic uptake of excreted products in the Southern North Sea, as determined by short-term kinetics. *Marine Ecology Progress Series,* 1 : 179-186.

Langeron, M., 1949. Précis de Microscopie. Masson et Cie, pp. 1430.

Larsson, U. & Hagström, A., 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Marine Biology,* 52 : 194-206.

Linley, E.A.S., Newell, R.C. & Bosma, S.A., 1981. Heterotrophic utilization of mucilage released during fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). I. Development of microbial communities associated with the degradation of kelp mucilage. *Marine Ecology Progress Series,* 4 : 31-41.

Lucas, M.I., Newell, R.C. & Velimirov, B., 1981. Heterotrophic utilization of mucilage released during fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). II. Differential utilization of dissolved organic components from kelp mucilage. *Marine Ecology Progress Series,* 4 : 43-55.

McHenery, J.G. & Birkbeck, T.H., 1982. Characterization of the lysozyme of *Mytilus edulis* (L.). *Comparative Biochemistry and Physiology,* 71B : 583-589.

McHenery, J.G., Birkbeck, T.H. & Allen, J.A., 1979. The occurence of lysozyme in marine bivalves. *Comparative Biochemistry and Physiology,* 63B : 25-28.

Romanenko, V.I., 1979. Bacterial growth at natural and low levels. *Archiv für Hydrobiologie, B., Ergebnisse der Limnologie,* 13: 77-84.

Schleyer, M.H., 1980. A preliminary evaluation of heterotrophic utilization of a labelled algal extract in a subtidal reef environment. *Marine Ecology Progress Series,* 3 : 223-229.

Sepers, A.B.J., 1977. The utilization of dissolved organic compounds in aquatic environments. *Hydrobiologia,* 52 : 39-54.

Strack, S., Bonotto, S. & Kirchmann, R., 1980. Radioactive contamination of the marine environment : uptake and distribution of ^{3}H in *Dunaliella bioculata*. *Helgoländer Meeresuntersuchungen,* 33 : 153-163.

Stuart, V., Lucas, M.I. & Newell, R.C., 1981. Heterotrophic utilization of particulate matter from the kelp *Laminaria pallida*. *Marine Ecology Progress Series,* 4 : 337-348.

VANDECASTEELE, C.M., 1981. Influence de la radiocontamination du sol sur l'activité de la microflore : comportement de *Rhizobium* et d'*Azotobacter* en présence du technetium-99. Mémoire de Licence. Laboratoire de Physiologie Végétale. Louvain-la-Neuve, septembre 1981, pp. 61.

VANDECASTEELE, C.M., DELMOTTE, A., ROUCOUX, P. & VAN HOVE, C., 1982. Technetium et organismes diazotrophes : toxicité, localisation, facteur de transfert. In : 'Environmental Migration of Long-lived Radionuclides', International Atomic Energy Agency, IAEA-SM-257/76, 275-286.

WILDUNG, R.E., MCFADDEN, K.M. & GARLAND, T.R., 1979. Technetium sources and behaviour in the environment. *Journal of Environmental Quality,* 8 : 156-161.

WOLTER, K., 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. *Marine Ecology Progress Series,* 7 : 287-295.

ZOBELL, C.E. & LANDON, W., 1937. Bacterial nutrition of the California mussel. *Proceedings of the Society for Experimental Biology and Medicine,* 36 : 607-609.

ZOBELL, C.E. & FELTHAM, C.B., 1938. Bacteria as food for certain marine invertebrates. *Journal of Marine Research,* 1 : 312-327.

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