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Experimental studies of trophic relationships between marine bacteria and bivalve molluscs

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Abstract

The importance of bacteria in the feeding of marine bivalves has been demonstrated by several authors. Some examples of good growth of molluscs were noted on a bacterial diet only. But, while the intense filtration of bacteria by molluscs has been observed, the exact role of bacteria in the nutrition of bivalves was not completely known. The filtered particles were sometimes eliminated as pseudofaeces, without any intestinal transit. On the other hand, live cells could also pass through the gut without being degested. To study in detail the fate of bacterial cells distributed as food to young bivalves, we used a new method which combines histology and scanning electron microscopy. This made it possible to observe, on serial histological sections of whole animals, the gut content and the condition of the ingested cells at the different levels of the intestinal tract. The ingestion and digestion by young mussels (*Mytilus edulis*) of some marine bacterial strains belonging to different taxonomic groups were studied by this method. Thus, partially digested bacterial cells were observed in the stomach, when the hind gut contained undamaged cells, three hours after food distribution. The results obtained for all the strains we tested are presented and discussed in this paper.

Introduction

The importance of bacteria in marine bivalves has not yet been fully evaluated. Many authors consider phytoplankton to be the essential dietary component, and this is regularly used to nourish bivalves in experimental laboratory cultures. In fact, however, these phytoplankton cultures also contain a large number of bacteria, dead cells and metabolic products of algae and bacteria.

Numerous studies have shown that phytoplankton is efficiently retained by the gill system of the bivales (JØRGENSEN 1949; DAVIDS 1964; MØHLENBERG and RIISGARD 1978). But the uptake and assimilation of various dissolved organic products, such as sugars and amino acids, have also been demonstrated (PEQUIGNAT 1973; WRIGHT and STEPHENS 1978; STEWART 1979). Several authors have demonstrated the growth of molluscs fed exclusively with bacteria cultivated in the laboratory (ZOBELL and LANDON 1937). Finally, the organic detritus on which bacteria can develop may also form part of the bivalve diet (NEWELL 1965; LENZ 1977). We only want in this paper to discuss the utilization of planktonic bacteria as a food source by the marine bivalves. The most common bacteria measure approximately one micrometer. However, in the Mytilidae, the bivalves capable of retaining the smallest particles, retention efficiency decreases markedly for particles of less than 2 μm diameter, (JØRGENSEN 1975).

In this case, the animals would only be able to retain either bacteria forming microcolonies of adequate size, or mucus bacterial agglomerates. Another important question is that of the digestibility of the bacteria. According to McHENERY and BIRKBECK (1979) *Mytilus edulis* possesses digestive tract lysozymes capable of degrading the glycopeptids which form an important part of the bacterial cell wall. Numerous microbiological studies of the accumulation of fecal bacteria by molluscs have partially answered these questions. First of all, bivales do ingest bacteria. But not all ingested bacteria are digested, as accumulation of cells capable of development in cultures is usually found (CABELLI and HEFFERNAN 1970).

We have also obtained similar results from studies of the commensal microflora of some bivalve species, and in particular *Mytilus edulis* (PRIEUR 1980).

The results obtained, using only bacteriological methods, show that there is not only ingestion and accumulation of living microorganisms, but also a modification of the bacterial population initially living in the surrounding sea water. Fermentative gram negative rods, like *Vibrio*, are particularly abundant in the animals. We have advanced several explanations of this phenomenon: selective ingestion of certain bacteria, varying resistance to the bivalve enzyme system, possible proliferation in the digestive tract.

Our experimental program was designed to answer the following questions: May bacterial cells be ingested by bivalves, and is there a component of selectivity?

Are the bacterial cells ingested?
At which points in the digestive tract may living bacteria be observed?

To approach these questions, we believed it necessary to use a precise method of observing bacteria in the digestive tract, as indirect methods of evaluating bacterial populations seemed insufficient. The techniques involving particle counts before and after ingestion do not allow an evaluation of the integrity of the cells after passage through the animals. The utilization of radioactively tagged bacteria also presents serious difficulties. The integrity of the bacterial cells before ingestion cannot be guaranteed. In such cases, part of the label is transformed into dissolved form and may be directly taken up by the animal. The labelled elements may also be found in the bacterial metabolic products before or during ingestion, and then in the molluscs' tissues, without having been digested. The complexity and fragility of the bivalve digestive tract do not allow the analysis of precise portions of the tract after dissection.

The technique we have used is a method of observation which precludes quantitative data. It is a combination of histology and scanning electron microscopy (PRIEUR 1980). It does, however determine with precision at which points in the digestive tract, and in what state the ingested particles are found. We present the results obtained with this method, which in our opinion constitutes an important complement to the methods previously described.

Methods

We used six bacterial strains isolated on 2216 E (OPPENHEIMER and ZOBELL 1952) OR TOBS (KOBAYASHI et al. 1963) agar, from sea water and ground *Mytilus*. Three of them were *Vibrio* type bacteria, one was a *Pseudomonas*, one was a gram positive rod, and the last one was a gram positive coccal bacterium.

From agar cultures, a bacterial suspension was prepared in sterile sea water, twice centrifuged and rinsed. It was resuspended in sterile sea water and distributed as food with a concentration of 2 million bacteria per ml in the experimental beaker. We used

small mussels, 6 to 10 mm in length, sampled in the bay of Brest. They were starved for one week in the laboratory in millipore filtered sea water, changed every day. For the experiment, a small number (6 to 12) of animals was placed in the experimental beaker filled with 0.2 μm millipore filtered sea water, at least one hour before bacterial food distribution. At regular times, 1 or 2 animals were sampled, and fixed in histological fixative liquid. After dehydration and paraffin embedding, sagittal and transverse sections were made. The sections were mounted both on glass slides for histology and on coverslips for scanning electron microscopy. The sections mounted on the glass slides were gram stained and observed in a light microscope to detect the position of food in the digestive tract. The coverslips, following the interesting sections were then prepared for SEM examination. After paraffin elimination in toluol, the sections were dehydrated in alcohol, alcohol-acetone 50 %, pure acetone, and then transfered to a critical point drying apparatus with liquid CO_2. After critical point drying, the coverslips were mounted on stubs, gold coated and observed with a scanning electron microscope (Jeol JSM 35) at an acceleration voltage of 35 Kv. The method is summarized in Figure 1.

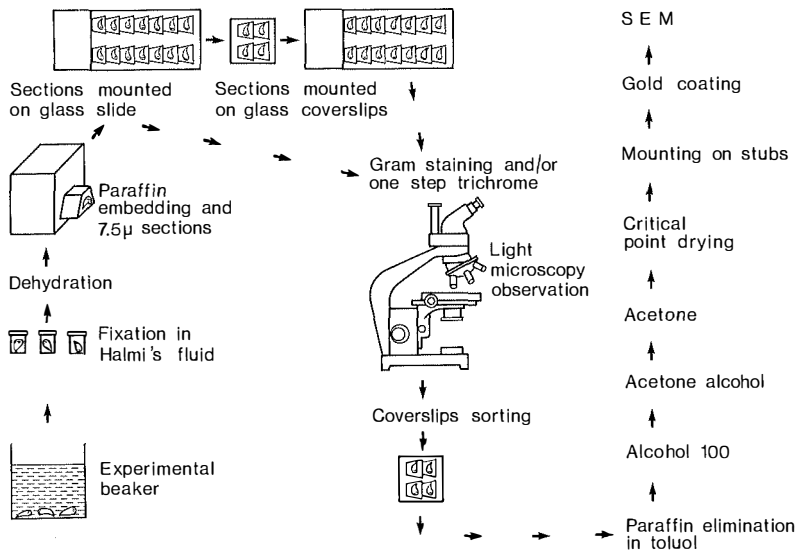

Figure 1
Processing of the samples for scanning electron microscopy

Results

A schematic representation of the digestive tract of *Mytilus edulis* is presented in Figure 2. The short oesophagus opens up to a voluminous stomach equipped with a caecum on the ventral side. The stomach is extended posteriorly by a double duct composed of a mid gut and a style sac, which are separated by a groove. The mid gut is continued by the hind gut, initially directed towards the front, and then towards the rear after a loop at the level of the digestive gland. This complex gland is formed by numerous ramifications of the diverticulum which opens up to the stomach.

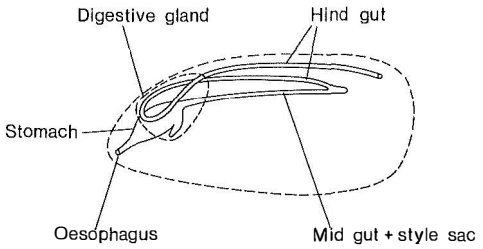

Figure 2
Organisation of the digestive tract of *Mytilus edulis*.

The distribution of food in the digestive tract is very rapid. 15 to 30 minutes after the addition of the bacterial suspension to the experimental system, the bacteria were located in the stomach (Plate 1, photograph 3, strain EM 142), in contact with the crystalline style (Plate 1, photograph 2, strain EM 142), and in the hind gut (Plate 1, photograph 1, strain EM 142).

No selectivity was noted in the ingestion of the bacterial strains prepared. One hour after the start of the experiment, the bacteria were observed at least at the level of the stomach, and they all appeared intact (Plate 1, photograph 4, strain ME 264).

The appearance of bacterial cells in the stomach clearly indicates that either they were agglomerated by mucus secreted by the animal (Plate 1, photograph 6, strain EM 142), or they formed microcolonies 4–6 μm in diameter (Plate 1, photograph 5, strain ME 300). This dimension is comparable to the size of small phytoplanktonic cells which are known to be efficiently ingested by Mytilidae.

Thus, the digestibility of bacterial cells has been demonstrated. The results are particularly clear-cut for the strains ME 256 (Plate 2, photograph 1), ME 300 (Plate 2, photograph 2), and EM 556 (Plate 2, photograph 3). During lysis, all these cells were observed at the level of the stomach, three hours after the start of the experiment. No lysis was observed in contact with the crystalline style on which intact agglomerated cells were found (Plate 1, photograph 2, strain EM 142). The method of digestion in the stomach is clearly extracellular.

It is interesting to note the simultaneous presence of bacteria in the stomach during digestion, and intact bacteria in the hind gut (Plate 2, photograph 5 strain ME 264; photograph 6, strain EM 142). On animals sampled 9 hours and 20 hours after the start of the experiment, the stomach and mid gut were found to gradually empty. In the hind gut, particles of indeterminate form appeared, conceivably waste material. However, intact bacterial cells were still observed, and this was particularly evident for *Vibrio* strains used (Plate 2, photograph 4, strain ME 283). There is rapid penetration of bacterial cells in the intestine, which do not stop at the level of the stomach. This may be due to an overly high concentration of available food. However, this undigested surplus was not immediately expelled, and this phenomenon may explain the accumulation of bacteria, well known among invertebrate marine filter feeders, and particularly the bivalves.

Conclusions

From a bacteriological point of view, bacterial cells suspended in sea water are really ingested by the mussels, by means of microcolonies or mucus agglomeration. Bacteria seem to be sensitive to the bivalve's enzymes, but the sensitivity is different

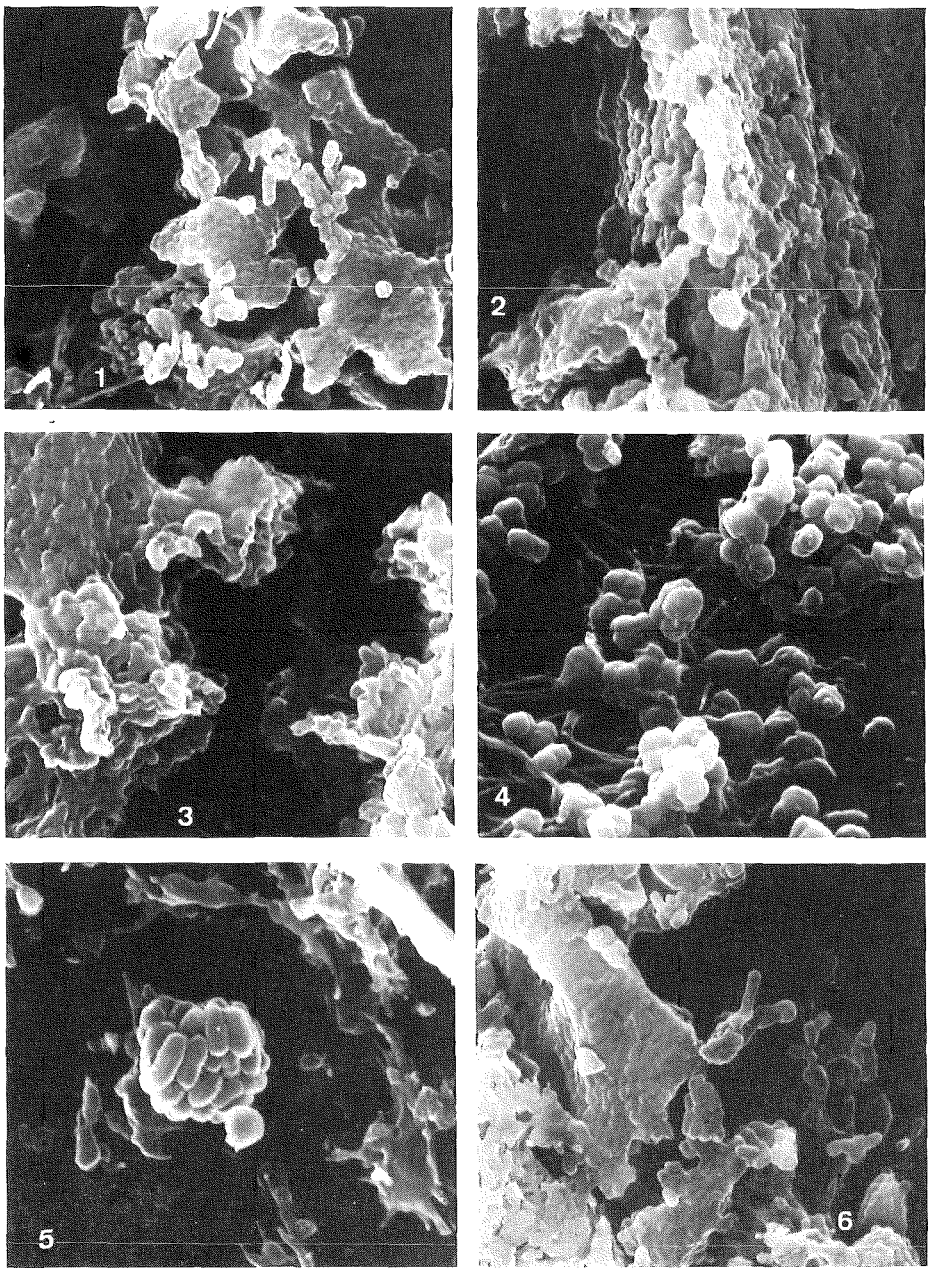

Plate 1

1. Strain EM 142 hind gut, 30 minutes after ingestion. x 3 500
2. Strain EM 142. against the crystalline style, 30 minutes after ingestion. x 3 500
3. Strain EM 142. in the stomach, 30 minutes after ingestion. x 5000
4. Strain ME 264. in the stomach, one hour after ingestion. x 3 500
5. Strain ME 300. microcolony in the stomach. x 6 300
6. Strain EM 142. bacterial cells agglomerated by mucus. x 3400

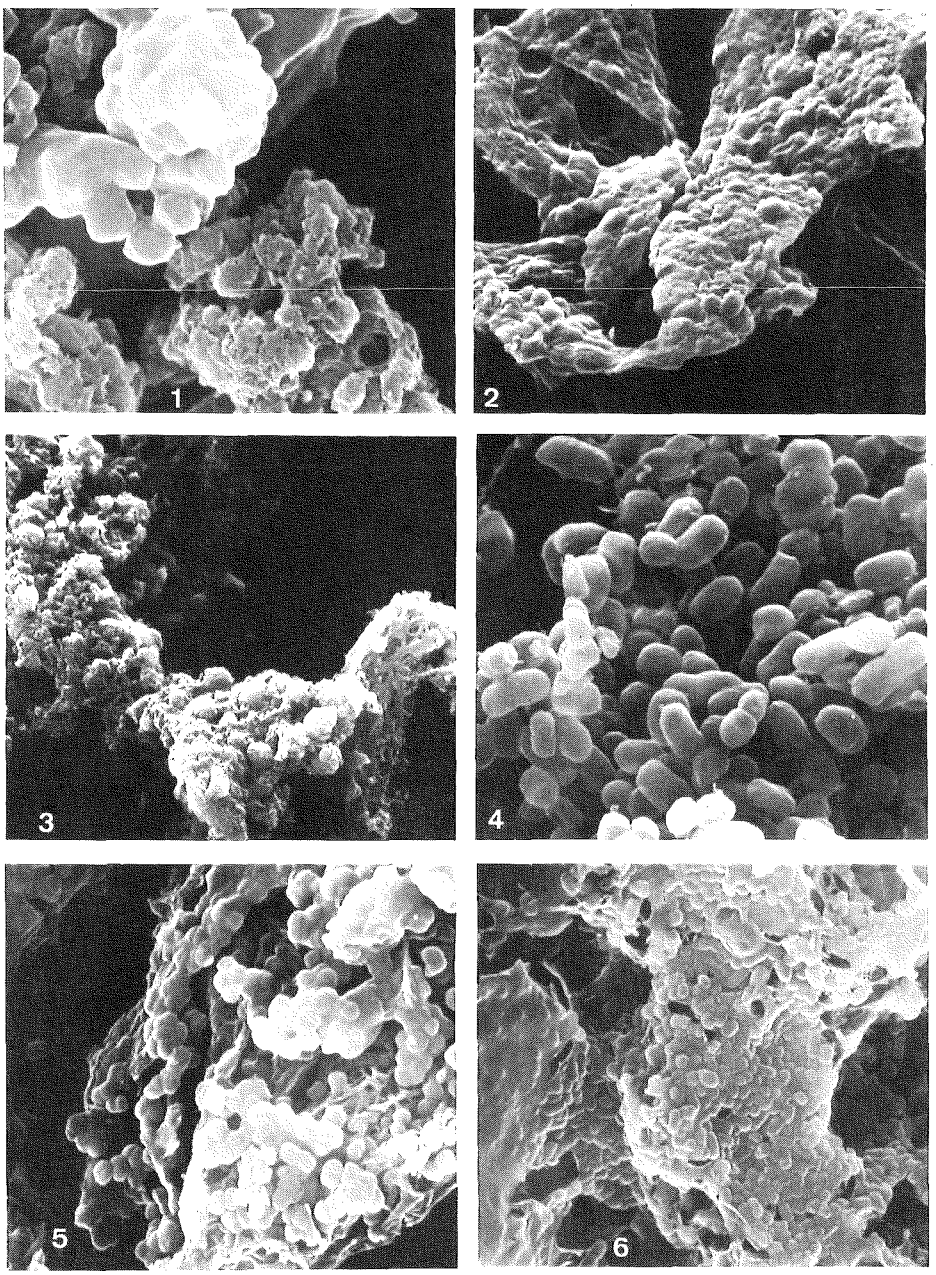

Plate 2

1. Strain ME 256. lysed cells in the stomach. x 5 700
2. Strain ME 300. lysed cells in the stomach. x 3 300
3. Strain EM 556. lysed cells in the stomach. x 2 600
4. Strain ME 283. normal cells in the hind gut, 20 hours after ingestion. x 5 700
5. Strain ME 264. normal cells in the hind gut, 1 hour after ingestion. x 2 900
6. Strain EM 142. normal cells in the hind gut, 1 hour after ingestion. x 3 800

from strain to strain. Bacteria can pass through the gut of the animal, and can remain several hours in this microbiotope. This is particularly evident for *Vibrio*.

From a malacological point of view, the ingestion of bacterial cells by bivalves is possible, but it is not a selective one. Extracellular digestion occurs in the stomach. Apparently living bacteria were observed on the crystalline style sac and the digestive function of this organ must be discussed. In our study, the food composed of identical particles was sorted in the stomach. One part went through the gut without digestion, while another part was digested in the stomach.

From an ecological point of view, the possibility of trophic relationships between marine bacteria and bivalve molluscs was demonstrated. During the time they remain in the gut, some bacteria, and particularly, the *Vibrio*, are apparently able to divide a number of times. If this phenomenon occurs, it would be an explanation of the presence of an important *Vibrio*-like population in the gut of bivalves compared to the bacterial population of sea water. It would also be a new parameter to consider in food chain models: bivales, and maybe other invertebrates, could be particular microbiotopes of important bacterial activity in the marine environment.

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