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# ROLE OF MICROBES IN THE NUTRITION OF SOME ESTUARINE AND MARINE BIVALVES

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## INTRODUCTION

BACTERIA in the sea are ubiquitous, although in the open sea they may be sparse. They are abundant in association with phytoplankton swarms, in estuarine mud and in turbulent waters. In spite of the high rate of multiplication they seem to maintain a constant level of density. It has been suggested that this might indicate that bacteria might be utilised as food by higher organisms.

Pourbaiz (1932) has shown that sponges ingest bacteria. Worms, oysters and cray-fish have been grown on a diet of bacteria. Gaarder and Spaark (1931) believed on the evidence of field studies that oysters do not feed on bacteria, but Wood (1953) reported that oysters could be fed on bacterial diet. This author has also recorded self-sterilisation of fish gut which he attributes to ingestion and digestion of bacteria (Wood 1953 & 1965). ZoBell and Feltham (1937) investigated the role of bacteria as food in *Mytilus californians*, *Emerita aneloga*, *Dendrostroma zostericola* and *Urechis caupo*. Rodina (1948) reported that normal growth rates in some fresh water molluscs could be obtained on a diet of bacteria. It seems to be fairly well recognised now that in filter-feeding animals bacteria are efficiently utilised as food. Precise quantitative studies alone can show how far the bacteria which are assimilated contribute to the growth of the organisms ingesting them. Zhukova (1963) investigated the role of bacteria as food in the polychaete *Nereis diversicolor*, the mollusc *Monodacna edentula* and the amphipod *Pontogammarus maeoticus*. He has also studied how far bacteria were assimilated and what proportion of the bacteria comprised the nutriment of these animals.

The present study records the observations on three species of bivalves *Arca granosa* (Linne), *Arca inaequivalvis* (Bruguière) and *Donax cuneatus* (Linne), which were grown on a diet of yeast, bacteria and diatoms.

## MATERIAL AND METHODS

Pure cultures of bacteria (*Micrococus candida*) and yeast (*Candida tropicalis*) isolated from marine zone of the Vellar estuary (11° 29'N. 79° 46'E.), were cultivated in the laboratory using the medium of the following composition :—peptone 1.0%, dextrose 4.0%, beef extract 0.3% and agar 1.5% prepared in filtered and sterilised sea-water. The centric diatoms *Melosira sulcata* (Ehr.) were grown in the medium described by Ramamurthy & Krishnamurthy (1965).

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Ten specimens of each of the bivalve species *Arca granosa*, *Arca inaequivalvis* and *Donax cuneatus* taken at random, were fed for an hour with pure cultures of bacteria and yeast separately. The intestine of each species was then cut into three portions (1) the descending limb of the intestine, (2) the coiled portion of the intestine and (3) the recurrent limb of the intestine. The microbial counts of the contents of each of these gut regions of each of the species were made by serial dilution of the gut contents using agar plate technique.

In the second series of experiment the aim was to determine whether the bivalves fed on the diet of bacteria, yeast and diatoms could increase in weight. Five specimens of each of the bivalve species were weighed separately correct to one milligram before the commencement of the experiment. The water content in the mantle cavity was removed with the help of a syringe, before weighing the specimen. The water adhering to the shell was removed by pressing the specimens between folds of a filter paper. The change in weight in each of the specimens was noted at intervals of a week during the period of the investigation.

The microbial suspension for feeding was prepared in aged, filtered and sterilised sea-water and adjusted to maintain a cell count of 500 million cells per ml. The cell count was made adopting the procedure described by Breed (1911). 600 ml. of the suspension was dispensed in separate glass troughs, in each of which one bivalve specimen was placed. The animals were maintained on the experimental diet for a month, but the medium was changed every day. Care was taken to maintain the cell count constant throughout the period of the experiment.

Controls in which the microbial food was omitted were run concurrently. In these controls five specimens of each species were kept in a glass trough containing 600 ml. of aged, filtered and sterilised sea-water. The medium was changed every day.

## Observations

In the first set of experiments the plate count gave a measure of the viable microbes in the different regions of the intestine. The counts showed that there was a progressive decrease of microbial population in the three successive regions of the intestine, indicating progressive increase in their utilisation. This would indicate that the experimental diets as they passed through the gut were digested (Table 1).

In all the feeding experiments, the weight of the specimen was recorded at intervals of a week as stated already. In Table II the percentage of increase in weight in relation to the initial weight is shown for weekly intervals and at the end of the month. The bivalves fed on a pure culture of a marine yeast showed the maximum increase in weight ranging between 20.2 to 32.2% of the initial weight. Those fed on bacteria showed an increase in weight ranging from 13.2 to 21.3% of the initial weight. Those fed on diatoms showed the minimum increase in weight, ranging from 4.5 to 10.5% of the initial weight.

The data relating to increments in weight after feeding were statistically tested adopting co-variance analysis and were found to be significantly different for each treatment at 5.0% level.

## Discussion

From the observations recorded above it is clear that bacteria, yeast and diatoms could be utilised as food by bivalves used in this experiment. As the bivalves fed

Table I

**Number of yeast cells and bacteria in successive regions of intestine*
(The numbers are expressed per gm. of intestinal content)

| Species | | Descending limb | Coiled portion | Recurrent limb |
|---|---|---|---|---|
| *A granosa* | Y | $6.6\times10^{8}$ | $4.1\times10^{8}$ | $2.6\times10^{8}$ |
| | B | $7.1\times10^{8}$ | $5.2\times10^{8}$ | $4.6\times10^{8}$ |
| *A inaequivalvis* | Y | $7.9\times10^{8}$ | $2.8\times10^{8}$ | $1.3\times10^{8}$ |
| | B | $8.2\times10^{8}$ | $6.3\times10^{8}$ | $5.1\times10^{8}$ |
| *D cuneatus* | Y | $8.9\times10^{8}$ | $3.0\times10^{8}$ | $1.1\times10^{8}$ |
| | B | $9.4\times10^{8}$ | $6.1\times10^{8}$ | $4.5\times10^{8}$ |

* (Mean of five replicates.) Y, Yeast; B, Bacteria

on yeast showed greater increase in weight than those on other diets, it would appear that yeast has greater food value than diatoms or bacteria. Next to yeast, bacteria are important in food value. The diet of diatoms contributed to least increment in growth. The diets showed also some differential effect on these bivalves. *Donax cuneatus* showed a greater percentage of increase in weight than the species of *Arca*. This may be due to the fact that the rate of filtration in *Donax cuneatus* is twice that in *Arca*, (Unpublished observations by Krishnamurthy). The two species of *Arca* however showed a similar increase in weight.

Rodina (1948) recorded an increase in weight upto sixty per cent in the fresh water bivalves, *Sphaerium*, *Musculum* and *Pisdium* fed on pure culture of bacteria and yeast, isolated from a lake. In the present investigation, however, the maximum increase in weight is 32.2% which is observed in specimen fed on yeast. Extensive comparative investigations would be required to generalise about the relative merits of microbial diets in fresh water and marine species. One thing is clear, that yeast, bacteria and diatoms can serve as sole source of food for the bivalves under laboratory conditions.

## Summary

Three species of bivalves, *Arca granosa*, *Arca inaequivalvis* and *Donax cuneatus* were grown on a pure culture of yeast, bacteria and diatoms for one month.

Bivalves fed on yeast showed greater increase in weight than those on other diets. The diets had also a differential effect on these bivalves. *Donax cuneatus* showed a greater percentage of increase in weight than *Arca*.

TABLE II

*Increment in weight of bivalves fed on different microbial diets*

*Donax cuneatus* (Linne)

| Food | Initial weight (mg.) | Increment in weight after 7 days | after 15 days | after 22 days | after 30 days |
|---|---|---|---|---|---|
| Yeast | 1179 (1032-1343) | 10.5% (9.1-11.3) | 15.1% (14.3-16.2) | 19.4% (17.8-21.0) | 32.2% (29.9-34.0) |
| Bacteria | 1106 (1022-1302) | 7.8% (6.3-9.4) | 14.2% (12.8-15.7) | 18.2% (16.9-19.7) | 21.3% (18.9-22.9) |
| Diatom | 1237 (1028-1348) | 3.9% (2.5-4.7) | 6.7% (5.1-7.9) | 8.3% (6.8-9.2) | 10.5% (8.0-13.0) |
| Control (animals were not fed) | 1152 (1003-1265) | —1.4% 0.8-1.6) | —1.6% (1.3-1.8) | —1.9% (1.4-2.1) | —2.1% (1.6-2.5). |

*Arca granosa* (Linne.)

| | | | | | |
|---|---|---|---|---|---|
| Yeast | 1382 (1285-1480) | 8.1% (7.2-9.8) | 12.2% (11.3-13.9) | 16.1% (14.8-17.3) | 20.2% (18.2-21.7) |
| Bacteria | 1446 (1394-1501) | 5.3% (4.1-6.4) | 7.4% (6.1-8.7) | 10.3% (9.2-11.8) | 13.2% (11.7-14.7) |
| Diatom | 1298 (1133-1421) | 1.2% (0.9-1.5) | 2.3% (1.8-2.6) | 3.3% (2.1-4.7) | 4.5% (4.1-5.0) |
| Control (animals were-not fed) | 1376 (1122-1487) | —1.2% (0.7-1.6) | —1.3% (1.1-1.5) | —1.7% (1.2-1.9) | —1.9% (1.7-2.2) |

*Arca inaequivalvis* (Bruguiere)

| | | | | | |
|---|---|---|---|---|---|
| Yeast | 1354 (1252-1432) | 10.2% (9.1-11.8) | 14.2% (13.1-15.3) | 18.3% (17.1-19.4) | 23.7% (19.9-26.4) |
| Bacteria | 1287 (1240-1305) | 6.3% (5.3-7.4) | 9.4% (8.1-10.7) | 12.0% (11.2-13.5) | 14.8% (13.4-16.1) |
| Diatom | 1501 (1403-1543) | 1.3% (0.8-1.5) | 2.4% (1.8-2.9) | 3.5% (2.3-4.8) | 4.6% (3.9-5.2) |
| Control (animals were-not fed) | 1489 (1305-1502) | —0.8% (0.6-0.9) | —1.2% (0.9-1.4) | —1.4% (1.3-1.6) | —1.8% (1.5-1.9) |

Figures within brackets indicate range of variation. *Mean of five replicates.

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