



Bacterial digestive enzyme activity in the stomach and hepatopancreas of *Meganyctiphanes norvegica* (M. Sars, 1857)

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Received 14 March 1993; revision received 6 October 1994; accepted 23 November 1994

Abstract

Northern krill, *Meganyctiphanes norvegica* were maintained in antibiotic-treated ("A"), and untreated (control, "C") aquaria. Each 24 h (for 72 h) numbers of saprophytic (Colony Forming Units, CFU), chitinolytic, and total bacteria (Acridine Orange Direct Count, AODC), were determined in the stomachs and hepatopancreata of animals. Parallely, the activities of chitinase, *N*-acetyl- β -D-glucosaminidase (NAGase), protease, cellulase and laminarinase were measured, and bacterial and endogenous chitinases compared by Fast Protein Liquid Chromatography (FPLC). CFU in "C" group stomachs were stable, but varied widely in the "A" group. AODC did not vary to any great extent. Hepatopancreas CFU decreased by four orders in the "A" compared to the "C" group. In "C" hepatopancreata, AODC were slightly higher than in "A". Numbers of chitinolytic bacteria fell steeply in the "A" group. Chitinase, NAGase, protease, and cellulase activities in the stomach were significantly lower (5% level) after 24 h in "A" than in "C". Laminarinase activity was significantly reduced in both organs after 72 h. Hepatopancreas chitinase and protease activities showed significant reductions only after 24 h. FPLC elution profiles of NAGase from krill, and bacteria grown on a medium semi-selective for chitinolytic bacteria differed. A number of bacterial chitinase peaks were absent in the "A" group. The API Zym system showed reduced proteolytic and lipase (C14) activity over 72 h. Endogenous bacteria are considered commensal with respect to the enzymes discussed.

Keywords: Bacteria; Chitinases; Digestive enzyme; Digestive organs; *Meganyctiphanes norvegica*

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1. Introduction

A wide range of studies of marine invertebrate digestive enzymes has been carried out (Mayzaud & Conover, 1975; Cox, 1981; Head & Conover, 1983; Klug et al., 1984; McConville et al., 1986; Mayzaud et al., 1987; Buchholz, 1989). The role of bacteria within the digestive tract of these animals in terms of enzyme production (Lewis & Whitney, 1968; Lewis, 1980; Mayasich & Smucker, 1987), however, has been comparatively overlooked.

A particularly high digestive potential is shown by crustaceans, and they can utilise a wide range of organic substrates. The euphausiid *Meganyctiphanes norvegica* for example is mainly carnivorous, but it can use phytoplankton as a staple food. As it generally feeds on copepods, chitin forms part of its diet, and its chitinase activity has been demonstrated and partly characterised (Spindler & Buchholz, 1988).

High numbers of bacteria are often found in the digestive tracts of animals but such associations are usually poorly understood or described. For example, bacteria are recognised for their role in cellulase digestion in termites but a similar relationship in the Antarctic krill, *Euphausia superba* Dana may be discounted (Turkiewicz et al., 1982). Conversely, Rakusa-Suszczewski & Zdanowski (1989) considered that high numbers of bacteria within the stomach of *E. superba* implies bacterial participation in digestive processes. In this respect bacteria in the crystalline styles of *Crassostrea virginica* produce chitinase and chitobiase, but are not essential for the well-being of the host (Mayasich & Smucker, 1987).

In order to investigate the contribution of bacteria to the digestive enzyme pool in the stomach and hepatopancreas of *M. norvegica* from the Kattegat, the activities of a range of digestive enzymes were compared in two groups of animals. An experimental group was maintained in an aquarium with broad spectrum antibiotics, and a control group kept with no antibiotics. In addition, a dye-labelled chitin derivative incorporated into a solid medium (Wirth & Wolf, 1990) was used to isolate chitinolytic bacteria. Exo- and endochitinases produced by these bacteria were compared chromatographically with those from whole krill homogenates. Furthermore, the activities of 19 constitutive enzymes were investigated with the semi-quantitative API Zym system.

2. Materials and methods

Northern krill, *M. norvegica* (M. Sars, 1857), (Crustacea, Euphausiacea) were caught with a ringtrawl (1 m² opening, 500 µm mesh) at a depth of ≈ 100 m in the Låsø Deep in the Danish Kattegat (57° 16' N, 11° 24' E) from FS *Alkor* between 15th and 17th October, 1991. Ten randomly selected live individuals were processed immediately for determination of bacterial numbers as described below, and a second group was frozen upon catching, –20 °C for 3 days, and thereafter stored at –80 °C. These animals sampled directly from the Kattegat were referred to as the “0” group.

2.1. *Experimental design*

Eighty to 90 live krill were transferred to two plastic tanks, each containing 50 l of fresh seawater, continuously aerated and maintained at 8 °C. One tank (group “A”) contained nystatin, 100 mg · l⁻¹, ampicillin trihydrate 500 mg · l⁻¹, and streptomycin sulphate, 250 mg · l⁻¹ according to Lewis (1980), whilst the second was untreated (Control group, “C”). No additional food was provided. After 24, 48, and 72 h, 10 animals were removed from each tank for time course parallel studies of bacterial numbers and enzymatic activity. Animals for the latter were immediately stored at -80 °C.

2.2. *Dissection of animals for bacteriological analyses*

Stomachs and hepatopancreata were aseptically excised from 10 live animals randomly selected from each group. Group “C” and “A” animals were net-caught from the aquaria to one litre of the same water in a sterile glass beaker: all animals, including those of the “0” group, were then transferred with ethanol flamed forceps to 30 ml of sterile seawater (30 s) for “washing” prior to dissection under a binocular microscope in sterile plastic Petri dishes, one dish per animal. Each was laid on its ventral surface, held across the carapace with sterile forceps, and an ethanol flamed mounted needle positioned immediately behind the eyes. A firm downward movement severed the head, which as pulled from the thorax frequently drew the stomach from the thoracic cavity. The stomach was collected with sterile forceps, weighed on sterile pre-weighed foil strips and transferred to an homogeniser in an ice bath. The hepatopancreas was removed by peeling back the carapace, weighed, and transferred to a second homogeniser, all aseptically.

Pooled stomachs and hepatopancreata were homogenised separately in 10 ml of filtered (0.2 µm) autoclaved seawater in a hand operated Potter homogeniser with a teflon shaft, maintained in an ice bath. A dilution series of each homogenate to 10⁻⁴ was prepared in sterile filtered and autoclaved seawater, and again maintained in an ice bath.

2.3. *Bacterial counts*

The numbers of saprophytic (CFU) and chitinolytic (Cht) bacteria in stomachs and hepatopancreata of the “0” group, and after 24, 48, and 72 h in the “C” and “A” groups, were determined by the spread plate method (100 µl of each dilution) in duplicate, on ZoBell’s 2216E Marine Agar (Difco), and CM-Chitin-RBV Agar (Wirth & Wolf, 1990) after 15 days incubation at 15 °C. The latter is semi-selective; chitinolytic bacteria are identified through their plaque production in the purple medium. CFU and chitinolytic bacteria in seawater at the collection site and in each aquarium were enumerated on these media (spread plate -100 µl) at the same temperature.

Total bacteria were determined by Acridine Orange Direct Count (AODC) in the 10⁰ homogenates used for viable bacteria in the “0” group, and after 24, 48, and 72 h in groups “C” and “A”. One hundred µl of each homogenate were added to 4.9 ml of

0.2 μm filtered formalin in 3.4% NaCl, to a final formalin concentration of 2%, and stored in sealed glass ampoules in a refrigerator until counting.

AODC in seawater and the “C” aquarium were determined in 9.5 ml of sample combined with formalin to 2%, and stored in sealed glass ampoules. High background fluorescence from undissolved antibiotics precluded AODC determinations for the “A” aquarium.

AODC were determined by epifluorescence microscopy according to Zimmerman & Meyer-Reil (1974), with a Carl Zeiss Jena FLUOVAL 2 microscope, fitted with an Apochromat HI 100/1.32; 160/0.17 objective, on black “Nuclepore” polycarbonate filters. All bacteria in a minimum of twenty fields (with a minimum of 0 and maximum of over 400 bacteria per field for water samples, and 0 to 40 in tissue samples) were counted (Cassel, 1965).

2.4. Chromatographic analyses of bacterial enzymes

The chromatographic properties of bacterial NAGase and chitinase were investigated in hepatopancreas and stomach derived isolates that had produced plaques on CM-Chitin-RBV. Several representative colonies were aseptically transferred to 200 ml of liquid medium containing 34 $\text{g}\cdot\text{l}^{-1}$ NaCl, 3.0 $\text{g}\cdot\text{l}^{-1}$ beef extract (Difco), 10 $\text{g}\cdot\text{l}^{-1}$ peptone (Difco), and 10 mg of purified chitin (Sigma C-3641), and incubated at 15 °C for 1 wk in a conical flask on an orbital shaker.

The culture was sonicated at 30% of maximum power three times for 15 s, with a break of 20 s between each (Branson, Sonifier B12, microstic 101-148-063). The suspension was centrifuged at 4000 g (Heraeus Minifuge 390) and the supernatant filtered successively through 3, 0.45 and 0.2 μm membrane filters (Sartorius). The filtrate was reduced to 5 ml with an ultrafiltration cell (Amicon, Type 8050, Membrane Diaflo PM 10) using a pressure of 3 bar. The material on the filter was rebuffered and applied to the chromatography column as described below for krill extracts.

2.5. Preparation of digestive organs for enzyme analyses

The stomach and hepatopancreas were sectioned from frozen animals and homogenised separately 1 ml of Citrate/Phosphate buffer (CPB), pH 5.5, by ultrasonication (3×5 s with a break of 15 s) with a Branson Sonifier B-12, Microstic 101-148-063, at 30% of maximum power.

2.6. Protein and enzyme activity measurements

Protein was determined in individual organ extracts according to Bradford (1976) using the BIORAD-microassay. Standards with Bovine serum Albumin (BSA) were run in parallel.

The activities of chitinase (EC 3.2.1.14) and NAGase (EC 3.2.1.30) in each stomach and hepatopancreas, and the FPLC fractions, were measured according to Saborowski et al. (1993) using CM-Chitin-RBV as chitinase substrate. Total protease activity was determined with azocasein-Na-salt as substrate (Serva 14391). The reaction mixture

contained 200 μl of 0.2 M CPB, pH 6.0 and 20 μl of sample. After preincubation at 35 °C for 5 min., the reaction was started with 50 μl of azocasein solution (1% in CPB). After 30 min incubation at 35 °C the reaction was stopped with 500 μl of trichloroacetic acid (TCA, 8% in dist. water). The precipitate was separated by centrifugation (5 min, 15000 g), and the absorption of the supernatant read at 366 nm in a spectrophotometer. Cellulase (EC 3.2.1.4) activity was determined with the dye labelled substrate CM-Cellulose-RBB (Wolf & Wirth, 1990), adapted to 1.5 ml reaction tubes. To 250 μl of 0.2 M CPB, pH 6.0, 50 μl of sample and 100 μl of substrate (4 mg/ml) were added to initiate the reaction. After incubation at 35 °C for 30 min, the reaction was stopped by addition of 100 μl 1 M HCl. After centrifugation (5 min, 15000 g), the supernatant's absorption was read at 600 nm. Laminarinase (EC 3.2.1.6) activity was determined essentially as described for cellulase, although CM-Curdlan-RBB (4 mg/ml) was used as the substrate, and the reaction terminated with 2 N HCl. CM-Cellulose-RBB and CM-Curdlan-RBB are available from Loewe Biochemica, Otterfing, Germany. All assays were run in duplicate with a parallel blank.

2.7. Influence of antibiotics on enzyme assays

In order to avoid misinterpreting the enzyme activity results, the influence of the antibiotics on the enzyme assays was determined. In place of the buffer, 10 μl of antibiotic solution at the experimental concentration were used in the assay. This volume corresponds to the maximal capacity of the stomach and hepatopancreas. Results of five assays per enzyme were compared with parallel assays run without antibiotics.

2.8. Chromatography

To distinguish endogenous *M. norvegica* N-acetyl- β -D-glucosaminidase (NAGase) and chitinase from bacterial enzymes, chromatographic properties of *M. norvegica* extracts and chitinolytic bacteria were compared. Chitinase and NAGase chromatography was carried out with a Fast Protein Liquid Chromatography (FPLC, Pharmacia) system on crude extracts of whole animals. Five animals of total wet weight 500–600 mg were homogenised in 2 ml 0.2 M CPB, using an ultraturrax (Janke and Kunkel, TP 18/10). Homogenisation was carried out on ice at maximal speed (20000 rpm), three times for 20 s, with a break of 20 s between each. The homogenate was then centrifuged at 80000 g (Heraeus Christ, Omikron, rotor 9420), and the supernatant desalted and rebuffered into 0.01 M Imidazole/HCl buffer, pH 6.8, using Sephadex-G25-PD10 columns (Pharmacia, 17-0851-01). The eluent was applied to a Q-Sepharose anionic exchange column with a bed volume of 10 ml, and elution effected with a 0 to 0.85 M NaCl gradient in 0.01 M Imidazole/HCl buffer, pH 6.8. Samples were separated into 93 fractions of 1.4 ml each. The protein profile was measured with a UV-detector (Pharmacia, Uvicord II) at 280 nm. Enzyme activities were measured in every second, and each fraction respectively, when activity peaks occurred.

2.9. API Zym assays

The semi-quantitative API Zym system (API bioMerieux, Marcy-letoile, France) was used to investigate the activity of 19 constitutive enzymes in the stomachs and hepatopancreata of animals from each group (Table 1).

Separate test strips were inoculated with the 10° stomach and hepatopancreas homogenates described above. The manufacturer recommends an incubation temperature of 37 °C over a period of 4 h, but such a temperature was considered inappropriate for boreal marine bacteria; strips were incubated for ten days at 15 °C. Controls “inoculated” with the sterile NaCl diluent were incubated alongside the test strips, and all processed in accordance with the manufacturer’s instructions. In contrast to the manufacturer’s instructions, however, results were classified as “no activity”, “reduced activity”, and “full activity”.

2.10. Statistics

Protein amount and enzyme activities in treated and untreated groups were compared pairwise for significant differences by the Mann-Whitney *U*-test. Results are presented as means with 95% confidence intervals.

Table 1
Enzyme activities in the stomach and hepatopancreas of *M. norvegica* determined with the APIZym assay

	Stomach								Hepatopancreas							
	0 h		24 h		48 h		72 h		0 h		24 h		48 h		72 h	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A		
1 Control	○	○	○	*	○	○	○	○	○	○	○	○	○	○	○	
2 Alkaline phosphatase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	●	
3 Esterase (C4)	◐	◐	●	*	○	●	◐	◐	●	◐	●	○	○	○	○	
4 Esterase lipase (C8)	●	●	●	*	◐	●	◐	●	●	●	●	●	●	●	◐	
5 Lipase (C14)	●	○	◐	*	○	●	●	●	●	●	●	●	◐	●	○	
6 Leucine arylamidase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	◐	
7 Valine arylamidase	●	●	●	*	◐	○	○	●	●	●	●	●	●	◐	◐	
8 Cysteine arylamidase	◐	◐	◐	*	○	○	○	●	◐	●	◐	◐	○	○	○	
9 Trypsin	●	◐	◐	*	○	○	○	●	●	●	●	●	○	○	○	
10 Chymotrypsin	●	◐	◐	*	○	○	○	●	○	○	○	○	○	○	○	
11 Acid phosphatase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	●	
12 Naphol-AS-Bi-ph. hydrol.	●	●	●	*	●	○	●	●	●	●	●	●	●	●	●	
13 α-galactosidase	◐	●	●	*	●	○	○	●	●	●	●	●	◐	●	●	
14 β-galactosidase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	●	
15 α-glucosidase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	●	
16 β-glucosidase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	●	
17 N-acetyl-β-glucosaminidase	●	●	●	*	●	●	●	●	●	●	●	●	●	●	●	
19 α-mannosidase	◐	●	●	*	●	◐	○	●	●	●	●	●	●	●	●	
20 α-fucosidase	◐	●	●	*	◐	◐	○	●	●	●	●	●	●	●	●	

● = full activity, ◐ = reduced activity, ○ = no activity, C = Control, A = Antibiotic treated, * = no data.

3. Results

3.1. Saprophytic bacteria (CFU) on marine agar

CFU in the “0” group numbered $\approx 1.3 \times 10^6 \text{ g}^{-1}$ (wet weight) of stomach tissue, and $1.7 \times 10^5 \text{ g}^{-1}$ (wet weight) of hepatopancreas (Fig. 1). No CFU were isolated from the stomachs of the antibiotic treated group (“A”) after 24 h, but they recovered strongly by 48 and 72 h. In the “C” group stomachs, bacterial numbers increased slightly over the duration of the experiment, attaining $5 \times 10^8 \text{ g}^{-1}$ by 72 h.

CFU were not fully eradicated from the “A” group hepatopancreata after 24 h, although their numbers were significantly reduced compared to the control group, and remained stable throughout the experiment.

Saprophytic bacteria in seawater at the catch site (100 m depth) numbered 33 cells per millilitre (Fig. 1). In the “C” aquarium water, CFU increased rapidly and by 48 h exceeded 1000 cells per ml. This level was maintained until the experiment ended (Fig. 1). No CFU were isolated from the “A” aquarium after 24 h, although numbers recovered strongly, such that after 72 h numbers equaled those in the “C” aquarium.

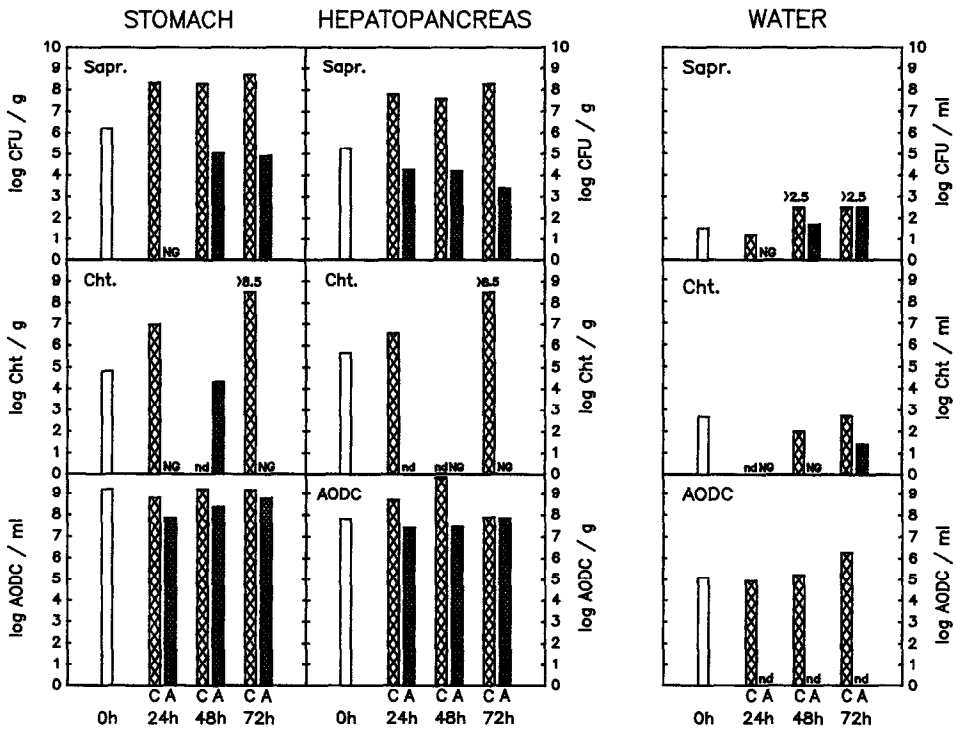


Fig. 1. Saprophytic bacteria (CFU) on ZoBell's 2216 Marine Agar, Chitinolytic bacteria on CM-Chitin-RBV agar, and Total bacteria (AODC) from *M. norvegica* stomach, hepatopancreas, and aquaria. At the start of the experiment (0 h) animals from the field and seawater were tested. NG = No Growth, nd = not determined.

3.2. Bacteria on CM-Chitin-RBV agar

In “0” group stomach’s and hepatopancreata, 10^4 to 10^5 chitinolytic bacteria per gramme wet weight were determined (Fig. 1). More chitinolytic bacteria were isolated from the hepatopancreas than from the stomach.

In the “C” group, numbers of chitinolytic bacteria in both organs increased towards the end of the experiment. Where tested, however, such bacteria were isolated on only one occasion from these organs in the “A” group (48 h, 2×10^4 g⁻¹).

The number of chitinolytic bacteria in seawater (“0”) exceeded by one order that of saprophytic bacteria. These bacteria were not enumerated in the control aquarium after 24 h due to a limited supply of the medium, although over 100 per ml were isolated after 48 and 72 h. No chitinolytic bacteria were detected in the “A” aquarium after 24 and 48 h, but they showed some recovery after 72 h. It should be pointed out, however, that counting on CM-Chitin-RBV medium was often difficult due to the large size of some plaques.

3.3. Total bacteria (AODC)

In the “0” group, AODC numbered 1.6×10^9 g⁻¹ of stomach and contents (wet weight), and 6.2×10^7 g⁻¹ of hepatopancreas (wet weight) (Fig. 1). In “C” group stomachs, AODC had fallen to 6.3×10^8 g⁻¹ after 24 h, but by 72 h compared with that noted in the “0” group. Fewer total bacteria were enumerated in the “A” group stomachs compared to parallel “C” group samples throughout the experiment, but their numbers increased with time, attaining 6×10^8 g⁻¹ (wet weight) after 72 h.

AODC in “C” group hepatopancreata showed an initial increase to 5×10^8 g⁻¹ before falling by 72 h to a level similar to that in the “0” group. Conversely, AODC in the “A” group fell initially by 24 h, but by 72 h also equaled that of the “0” group.

Total bacteria in seawater (“0”) numbered $\approx 10^5$ ml⁻¹. At 24 h, AODC in the “C” aquarium numbered only 8.5×10^4 ml⁻¹, but increased by 72 h to 1.8×10^6 cells·ml⁻¹, most of which were large rods. AODC in the “A” aquarium were not determined; undissolved and fluorescing antibiotic covered the filters supporting the samples.

3.4. Protein content and enzyme activities

Protein levels in the stomach increased in both “C” and “A” groups after 24 and 48 h compared to the “0” group, but returned to “0” group levels by 72 h (Fig. 2). Protein content in the hepatopancreas was relatively stable, with a slight increase towards the end of the experiment.

Enzyme activities in both groups decreased markedly over the duration of the experiment compared to those determined in the “0” group (Fig. 2). Largest decreases were shown by NAGase and cellulase in the stomach. Laminarinase activity in the “C” group stomach significantly exceeded that in the “A” group only at 72 h.

In “A” group stomachs, chitinase, NAGase, protease, and cellulase activities were significantly lower after 24 h compared to the “C” group. In addition, significantly lower chitinase and protease activities were detected in “A” group hepatopancreata after

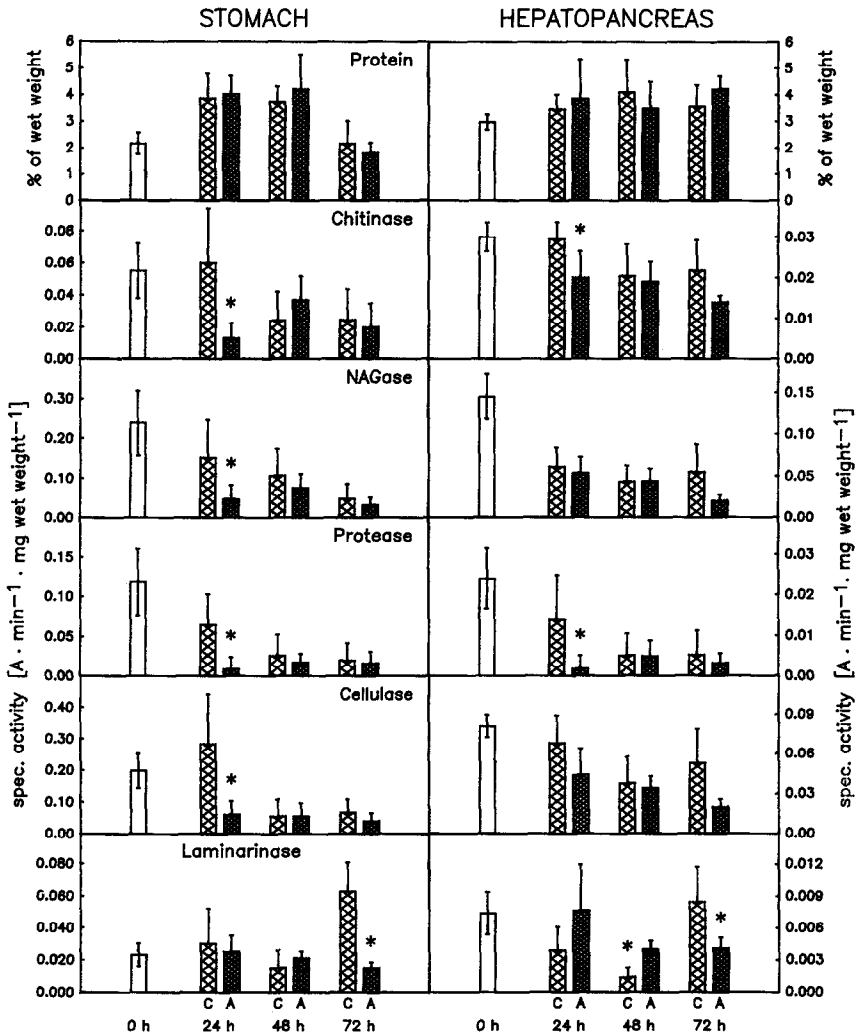


Fig. 2. Amount of protein and specific activities of selected enzymes in *M. norvegica* stomach and hepatopancreas in “0”, “C”, and “A” groups. Error bars represent 95% confidence limits of the mean ($n = 8-10$). *, significant differences between treatments, $P > 0.05$.

24 h. Laminarinase activity in the hepatopancreas was significantly lower in the “C” group after 48 h, but after 72 h the converse applied.

3.5. Chromatographic analyses

The FPLC elution profiles of *M. norvegica* NAGase (from groups “A” and “C”) showed two distinct activity peaks at fractions 40 to 55, and 80 to 90 (Fig. 3). Neither coincided with the single peak of bacterial NAGase. In contrast, the chitinase profile

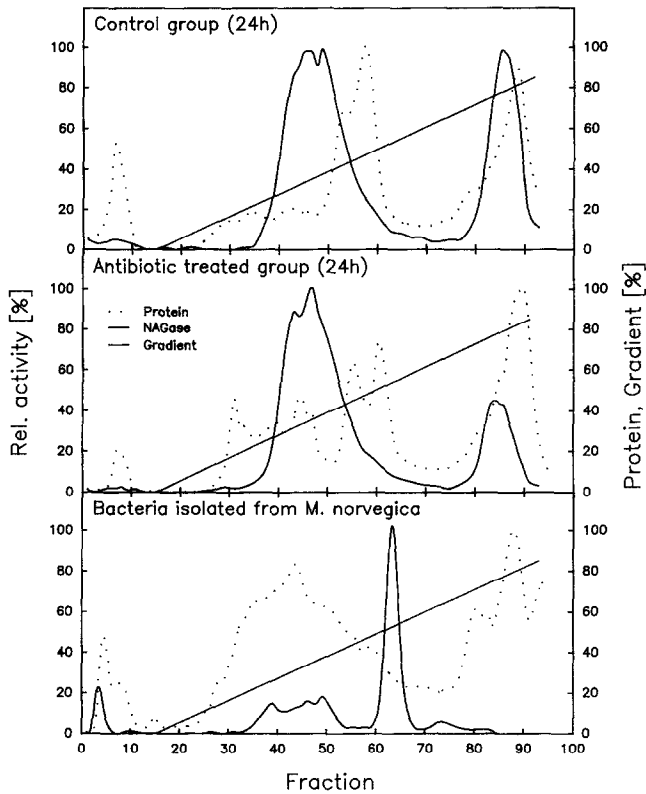


Fig. 3. FPLC elution profiles of NAGase from *M. norvegica* ("C" and "A" groups at 24 h), and from bacterial isolates.

of *M. norvegica* was split into several peaks which overlapped with the broad peak shown by bacterial chitinase in the fraction range 50 to 70 (Fig. 4). In the "A" group, fewer activity peaks were observed than in the "C" group, and those between fractions 40 to 53, and 75 to 80 were absent.

3.6. Influence of antibiotics on enzyme assays

In some cases the antibiotics influenced the enzyme assays. Significant differences occurred in the stomach with cellulase (98%), chitinase (105.8%), and protease (110.9%) compared to assays without antibiotics. Similarly, in the hepatopancreata differences were of the order of 95.3% for cellulase, and 126.9% for protease. Recalculation of the original data taking such effects into consideration did not affect significances, however, and the original data sets remained unchanged.

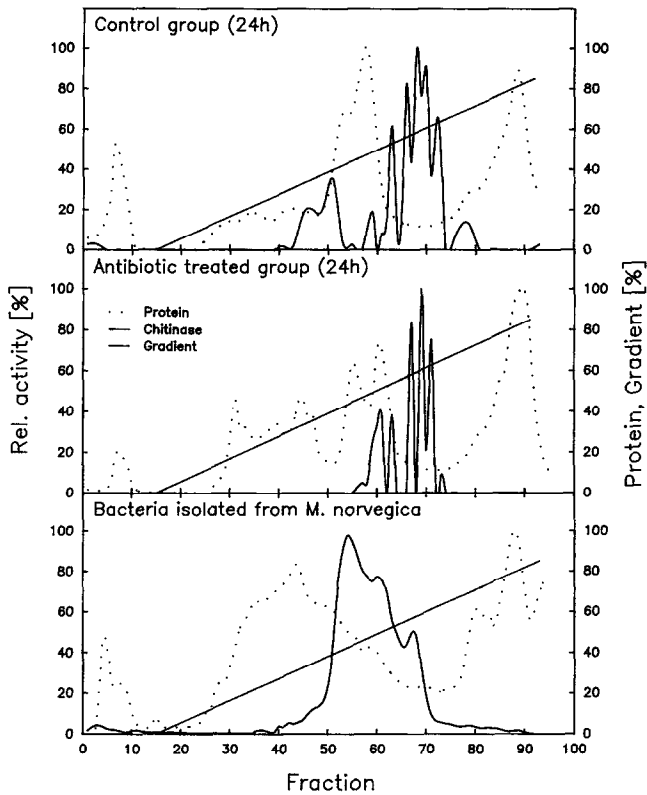


Fig. 4. FPLC elution profiles of chitinase from *M. norvegica* (“C” and “A” groups at 24 h), and from bacterial isolates.

3.7. API Zym analysis

The API Zym system revealed one major difference between treated and untreated animals in that no lipase (C14) activity was noted in the hepatopancreata of the “A” group after 72 h (Table 1); in “C” group hepatopancreata, full activity was recorded. In both groups a number of proteolytic enzymes such as trypsin, chymotrypsin, valine- and cysteine arylamidases showed a cessation of activity over the duration of the experiment. In the stomach, decreases in such enzyme activities were evident after 24 to 48 h. Antibiotic treatment did not affect most of the glycosidal enzymes, such as β -galactosidase, α - and β -glucosidase, and *N*-acetyl- β -glucosaminidase.

4. Discussion

The population of *M. norvegica* in the Låsø Deep has been extensively described (Boysen & Buchholz, 1984; Buchholz & Prado-Fielder, 1987; Buchholz & Boysen,

1988). This paper, however, describes the first complementary study of its bacteriology and digestive enzymes.

Both krill stomach and hepatopancreas host considerably higher numbers of CFU and total bacteria than the surrounding seawater. Numbers were in fact of the same order as found in *Penaeus aztecus* by Dempsey et al. (1989). Whether or not, in the case of the stomach, this results from the animal's feeding strategy, and/or in situ bacterial growth has not been determined; the former may concentrate cells in the stomach, but growth may also result from the elevated nutrient levels in the stomach compared to the seawater. The incidence of chitinolytic bacteria, however, may be partially explained by their association with the integument of copepods (Sieburth, 1979, p. 40) that are consumed by *M. norvegica*: copepods constitute 95% of the Kattegat zooplankton standing stock throughout the year (Buchholz & Prado-Felder, 1987).

Decreases in chitinase, NAGase, protease, and cellulase activities in the stomach, and in chitinase and protease in the hepatopancreas were both most significant in the antibiotic treated group after 24 h. This coincided with the most pronounced reductions in bacterial numbers. Bacterial recovery thereafter may have resulted from the proliferation of resistant strains. Activity reductions of most enzymes over the course of the experiment may have resulted from starvation, as noted in *Euphausia superba* (Saborowski & Buchholz, pers. comm.). Considering the animals were not fed this is certainly the most plausible explanation. Lowest enzyme activities were in most cases noted in the "A" group, and again these coincided with the lowest numbers of bacteria. In the "C" group, however, bacterial numbers did not correlate with the enzyme activities in either organ. One may have expected the recovery in bacterial numbers (saprophytic and chitinolytic) to result in increased enzyme activities, but one can argue that reduced nutrient supplies also lead to a decrease in the induction of bacterial enzymes: this would conflict with the fact that bacterial growth continued in what appeared to be nutrient limited conditions.

A bacterial contribution to chitinase activity in *M. norvegica* was demonstrated by the FPLC elution profiles (cf. Fig. 4). the absence of certain peaks in the "A" group chromatogram and their presence in that of bacterial chitinase describes the potential bacterial role in this respect. In the absence of growth of chitinolytic bacteria after 24 h, that chitinolytic activity that persisted must be considered of endogenous origin. In contrast to our results, however, Dempsey & Kitting (1987) noted no chitinolytic activity in bacteria isolated from penaeid shrimps. Furthermore, our FPLC work detected no bacterial NAGase activity in *M. norvegica*: Krill NAGase in fact occurs in two isoforms, one eluting at fraction 40 to 55 considered to be a moulting enzyme, the other eluting at fraction 85 representing the digestive enzyme (Peters, pers. comm.). The prominent bacterial NAGase peak does not overlap with any of those shown by the krill, and there was no significant difference between antibiotic treated and untreated animals.

With respect to the elution profiles of bacterial enzymes, it should be borne in mind that these were prepared from several colony types in one batch culture. The growth dynamics of such a culture may not reflect that of the in vivo bacterial population, nor the in vivo significance of such enzymes. These FPLC results were confirmed by the

API Zym system, within which no reduction in NAGase activity was recorded in the antibiotic treated animals.

Bacteria are involved in proteolytic activity in both the stomach and hepatopancreas in *M. norvegica*. This is evidenced by parallel reductions in total protease activity and CFU after 24 h. Such a contribution is further implied by the prominence of extracellular proteolytic activity in almost all groups of bacteria isolated from the Antarctic krill *E. superba* for example (Kelly et al., 1978; Zdanowski, 1981, 1988; Turkiewicz et al., 1982; Especje et al., 1987). Furthermore, decreased lipase (C14) (API Zym) activity in “A” group hepatopancreata is well demonstrated, with reductions in α -mannosidase and α -fucosidase activities being noted in the “A” group compared to the “C” group after 72 h. The question of why protease and cellulase activities did not increase in “C” group animals, within which bacterial numbers increased, remains unanswered.

With respect to laminarinase, no significant change in activity was noted in either organ after 24 h. By 72 h though, that in the “C” group stomachs exceeded that in both the “0” and “A” animals, an increase that did correspond with elevated numbers of bacteria. As suggested earlier, higher numbers of bacteria may well produce higher amounts of enzyme, regardless of the feeding conditions available to the host, and as such may account for the increase in laminarinase activity in the untreated animals.

The application of API Zym strips offered the possibility to measure a wide range of enzymes simultaneously. The results highlighted a decrease in lipase (C14) activity in “A” group hepatopancreata. Furthermore, reductions in α -mannosidase and α -fucosidase were also noted in the “A” group after 72 h. Most notably, proteolytic enzyme activities are reduced in both the stomachs and hepatopancreata of the “C” and “A” groups, which tie in with both reductions in bacterial numbers and food availability.

Although the scale of the role of bacteria in the *M. norvegica* digestive organs discussed has not been fully described here, the work confirms the involvement of bacteria in the production of a range of digestive enzymes. None of the enzymes considered, however, are likely to be solely of bacterial origin. In contrast, NAGase has been shown to be solely endogenous in origin. In this respect these bacteria must be considered as commensals within these organs in *M. norvegica*. A bacterial role in the production of proteolytic enzymes seems to be of some importance though, and this must form the basis for future studies. Some difficulty arose in interpreting the enzyme activity results because the actual loss in activity due to starvation could not be quantified: a more complex experimental design would clarify such an aspect. It will also be necessary to investigate the mechanisms that lead to the concentration of bacteria throughout the digestive tract before the role of bacteria therein can be fully described and understood.

Acknowledgements

We thank the crew of FS *Alkor* for their excellent work and assistance. The first author is indebted to the director of “Institut für Meereskunde”, Prof. D. Adelung for the invitation to the IfM. To Prof. S. Rakusa-Suszczewski and Dr. M. Zdanowski for

many hours of discussion, and Dr. D. Lewis for valuable comments on an earlier manuscript. The work was supported by a grant from the German Research Council (DFG Bu 548/2).

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