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Toxicity of bacteria towards haemocytes of *Mytilus edulis*

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Abstract — Haemocytes of *Mytilus edulis*, allowed to attach to the plastic surface of a tissue culture plate in the presence of haemolymph, were observed by time-lapse video recording. When bacteria were added at concentrations of 10 or 50 bacteria per haemocyte, certain bacterial strains caused rounding of the cells within 2–3 h. Haemolymph was necessary for the rounding to occur; if bacteria were added in sterile seawater there was no significant difference in the number of rounded cells between control and bacteria-treated cultures for up to 4 h. The haemolymph factor required for this activity was active at 1/64 dilution in seawater, was sensitive to trypsin treatment, and activity was halved on heating at 56 or 100 °C for 30 min. For the most toxic bacteria tested, *Vibrio alginolyticus* NCMB 1339 and *Vibrio anguillarum* A7, haemocyte cell rounding appeared to be induced by a very small number of bacterial cells. Bacteria-free culture supernatant of *V. anguillarum* 2981 induced rounding of haemocytes in a dose-dependent manner, with 50 % of cells being rounded at a dilution of approximately 1/500 of the culture supernatant. In a survey of 226 bacterial isolates, those isolated from incidents of disease in a bivalve hatchery were significantly more toxic towards haemocytes than bacteria isolated from hatcheries without disease or from turbot hatcheries. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

***Mytilus edulis* / *Vibrio anguillarum* / *Vibrio alginolyticus* / vibriosis / vibrio–haemocyte interaction / bivalve larvae**

Résumé — Toxicité bactérienne envers des hémocytes de *Mytilus edulis*. Les hémocytes de la moule *Mytilus edulis* mis en culture, sur des plaques en plastique pour cultures tissulaires, en présence d'hémolymphe, ont été observés par vidéo. Lorsque des bactéries sont ajoutées, à la concentration de 10 à 50 bactéries par hémocyte, certaines souches bactériennes provoquent l'arrondissement des cellules dans les 2 ou 3 h. L'hémolymphe est nécessaire à l'arrondissement des cellules ; si des bactéries sont ajoutées en eau de mer stérile, il n'y a pas de différence significative dans le nombre de cellules rondes, entre les cultures témoins et celles qui contiennent des bactéries, pendant 4 h. L'hémolymphe, utilisée pour ce test, est active à une dilution de 1/64e en eau de mer, elle est sensible à un traitement à la trypsine, et l'activité est réduite de moitié lors du chauffage à 56 ou 100 °C pendant 30 min. Pour la plupart des bactéries toxiques testées, *Vibrio alginolycus* NCMB 13339 et *Vibrio anguillarum* A7, l'arrondissement des cellules hémocytaires semble être induit par un très petit nombre de cellules bactériennes. Les cultures de *Vibrio anguillarum* 2981 exemptes de bactéries provoquent l'arrondissement des hémocytes en fonction de la dose appliquée, avec 50 % de cellules arrondies pour une dilution de 1/500e environ du surnageant en culture. Lors d'une étude de 226 bactéries isolées, celles isolées provenant d'incidents pathologiques en écloserie de bivalves étaient significativement plus toxiques envers les hémocytes que les bactéries isolées d'écloserie ne présentant pas de maladie ou provenant d'écloserie de turbots. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

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1. INTRODUCTION

The increasing worldwide demand for shellfish and the depletion of natural populations due to overfishing, falling fecundity and infectious diseases [5] have led to culture of larvae of many bivalve species in hatcheries. Although the hatchery culture of shellfish larvae is a well-established process, losses often occur owing to bacterial infection [6, 9, 21, 22]. Since the early work of Tubiash et al. [31] many studies have implicated vibrios as pathogens of bivalve larvae and the disease has been reproduced experimentally, for example, in larvae of the eastern oyster, *Crassostrea virginica* [10] and *Pecten maximus* [22].

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Table I. Ability of various bacteria to cause rounding of *Mytilus edulis* haemocytes.

Bacterium	Source	Reference*	Cell rounding** induced
V. anguillarum 2981	oyster, *Ostrea edulis*	[a]	H
V. anguillarum 4579	oyster, *Ostrea edulis*	[a]	H
V. anguillarum 5679	oyster, *Ostrea edulis*	[a]	M
V. anguillarum B1	oyster, *Crassostrea gigas*	[12]	M
V. alginolyticus NCMB 1339	clam, *Mercenaria mercenaria*	[31]	M
V. anguillarum NCMB 6	cod, *Gadus morrhua*	[2]	M
V. anguillarum 1197	saithe, *Pollachius virens*	[b]	L
V. anguillarum 91079	turbot, *Scophthalmus maximus*	[15]	M
Alteromonas 1-1-1 (NCMB 2024)	sand	[34]	L

Haemocytes were allowed to adhere to the surface of 24-well tissue culture plates and, after removal of non-adherent cells, were exposed to washed bacterial cells (10 per haemocyte) in *M. edulis* haemolymph. After 150 min at 20 °C the percentage of rounded cells was determined by microscopy.
* Source of bacterial isolates: [a] Professor B. Austin, Herriot-Watt University, Scotland; [b] Dr T. Hastein, National Veterinary Institute, Oslo, Norway.
** Isolates were classed as being of high (H), medium (M) or low (L) toxicity if they caused rounding of 67–100, 34–66 or ⩽ 33 % of cells, respectively, the total cell number being normalized to take account of cell rounding occurring in control, untreated samples.

The mechanisms by which vibrios cause disease in larvae are not fully understood but Elston [9] showed that different vibrios caused three different patterns of disease in experimentally infected larvae. One disease pattern appeared to be toxin-mediated, and in hatcheries vibriosis usually involves a drop in food consumption, cessation of swimming and settling of larvae to the bottom of the rearing tank followed by death. The mechanisms of toxin-mediated death have been investigated by Nottage et al. [26] who substantiated earlier reports of the involvement of both heat-stable toxins [8] and heat-labile toxins [7, 16] which were lethal to the larvae. The heat-labile toxin was characterized as a protease of 38–41 kD [23] and its production was demonstrated during infections of oyster larvae [24]. The heat-stable toxin, a low molecular weight (< 1 kD) factor with lethal and ciliostatic activity [26], was considered the principal factor involved in cessation of swimming and feeding activities which are characteristic of the sudden collapse of larval cultures [10].

Another toxic mechanism was suggested from the observation that washed cells of certain *Vibrio* strains rapidly inhibited filtration by adult mussels, *Mytilus edulis* [19]. This was not correlated with the degree of binding of such bacteria to the gills of the bivalves [4]. However, at some stage during infection or during the processing of micro-organisms, bacteria will come into contact with phagocytic cells of bivalves. In a preliminary investigation of the interaction of vibrios with *M. edulis* at the cellular level, it was shown [25] that differences in virulence of two strains of *V. alginolyticus* for oyster larvae were reflected in differences in toxicity of washed bacterial cells to haemocytes of *M. edulis*. The ratio of bacteria to haemocytes used was high (up to 500:1) and the assay was based on uptake of the vital stain neutral red [25]. In this study we show that *M. edulis* haemocytes which have been allowed to spread on glass slides or plastic surfaces become rounded when exposed to certain bacteria. The effect of time of incubation, bacteria/haemocyte ratio, and requirement for haemolymph have been investigated to develop a simpler assay to study the interaction of bacteria with bivalve haemocytes.

2. MATERIALS AND METHODS

2.1. Bacteria

Previously characterized bacteria and their sources are shown in *table I*. In addition, 226 isolates from episodes of vibriosis in a hatchery at Seasalter Shellfish, Reculver, Kent (52 isolates), from normal *C. gigas* larvae at CEFAS, Conwy, Gwynedd, Wales (8 isolates), Guernsey Sea Farms, Guernsey (23 isolates) and various turbot hatcheries (143 isolates [20]) were also tested (*table II*).

Overnight cultures of bacteria, shaken in baffled 250-mL Ehrlenmeyer flasks at 100 rpm at 25 °C in Difco Marine Broth or Oxoid Nutrient Broth No. 2 supplemented with 1.5 % (w/v) NaCl were collected by centrifugation, washed in 3.2 % sterile saline, standardized with sterile 3.2 % saline to A600 = 1 (approximately 1×10^9 cell mL^{-1}). The cells were collected by centrifugation, washed twice with sterile saline and resuspended to their original volume in saline, 0.45-μm- (pore diameter) filtered haemolymph or 0.45-μm-filtered seawater. Bacteria-free supernatants of cultures of *V. anguillarum* 2981 and other bacteria were prepared by centrifugation of 72-h Marine Broth shake cultures (100 rpm at 25 °C), followed by filter sterilization (0.22-μm filter).

2.2. Mussels

Adult *M. edulis* (ca. 50–70 mm in shell length) were obtained locally and were maintained in tanks of aerated seawater at 10 °C. Prior to use they were acclimated at room temperature for 1 h.

Table II. Toxicity of marine bacteria to haemocytes of *Mytilus edulis*.

Source of isolates	Number of isolates in the following toxicity categories*		
	High	Medium	Low or non-toxic
Standard bacteria (see *table I*)	2	4	3
Seasalter, Whitstable	13	10	29
CEFAS, Conwy	1	1	6
Guernsey Sea Farms	–	8	15
Various turbot hatcheries	4	30	109
Total	20	53	162

* Isolates, tested at a bacteria/haemocyte ratio of 10:1, were classed as being of high, medium or low toxicity if they caused rounding of 67–100, 34–66 or 0–33 % of cells, respectively, the total cell number being normalized to take account of cell rounding occurring in control, untreated samples.

2.3. Haemolymph, haemocytes and preparation of haemocyte monolayers

Haemolymph was obtained from the posterior adductor muscle of *M. edulis* using a 1-mL syringe and a 26-g needle. Immediately after collection, haemocyte monolayers were prepared by adding 100 μL of cell suspension to each well of a 24-well flat-bottomed microtitre plate and allowing the haemocytes to adhere for 1 h at room temperature. Non-adherent cells, estimated to be less than 10 % of the total, were removed by washing twice with filter-sterilized seawater. The cell concentration in haemolymph was determined immediately after collection using a Neubauer chamber, and this concentration was used to determine the number of bacteria to be added in the haemocyte toxicity assay. Haemolymph, collected from five mussels, was centrifuged, filtered (0.45 μm) to remove cells and used as a final diluent for the washed suspension of test bacteria.

2.4. Haemocyte toxicity assay

Haemocyte monolayers were exposed to washed bacteria, at concentrations equivalent to 10, 50 or 500 bacteria per haemocyte, suspended in either filtered haemolymph or filter-sterilized seawater. Control haemocytes were overlayered with the above fluids without bacteria. The plates were incubated for 3 h (unless otherwise stated) at 20 °C and the proportions of spread and rounded haemocytes determined from photographs obtained using an Olympus CK2 inverted microscope and camera attachment. One-hundred cells were counted and results were normalized to take into account the fraction of rounded cells in control samples, using the formula: cell rounding = $(T - C)/(100 - C) \times 100$, where T is the percentage of rounded cells in the test sample, and C is the percentage of cells rounded in control samples.

The toxicity of culture supernatants to haemocytes was determined by preparing 1-mL serial doubling dilutions of filter-sterilized (0.22 μm) 72-h culture supernatants of *V. anguillarum* 2981 in filter-sterilized seawater. These were added to the haemocyte monolayers and the proportion of rounded cells determined after 3 h at 20 °C.

To inactivate bacteria, suspensions of washed cells of *V. anguillarum* 2981, at a concentration of 1×10^9 bacteria mL^{-1}, were treated overnight with 1 % formaldehyde in 3.2 % saline, washed in saline and resuspended to 1×10^9 bacteria mL^{-1} before treatment with filter-sterilized haemolymph.

In experiments to determine the effect of haemolymph concentration on haemocyte rounding the filtered haemolymph was diluted in filter-sterilized seawater for use in the assay.

Trypsin treatment of filtered haemolymph was carried out with trypsin (Sigma, Poole, UK) at a final concentration of 50 μg mL^{-1} for 1 h at 25 °C, after which *V. anguillarum* 2981 was suspended in the trypsin-treated haemolymph and the toxicity to haemocyte monolayers determined.

2.5. Kinetics of haemocyte rounding

Time-lapse recording was used to monitor changes induced by bacteria in 13 experiments to determine the kinetics of the rounding process, after which still photography was used for subsequent experiments. Time-lapse recording was carried out using a Leitz Diavert inverted microscope with a Panasonic video camera and Panasonic Super VHS model AG7620 video recorder operating at 1/80th of real time. Still photographs taken at specified intervals were used to determine the percentage of cells rounded as described above (Haemocyte toxicity assay).

2.6. Statistical analysis

The χ^2 test [33] was used to determine the significance of differences between groups of bacteria in their ability to induce haemocyte rounding. The Student's *t*-test and other statistical tests were also carried out as described by Wardlaw [33].

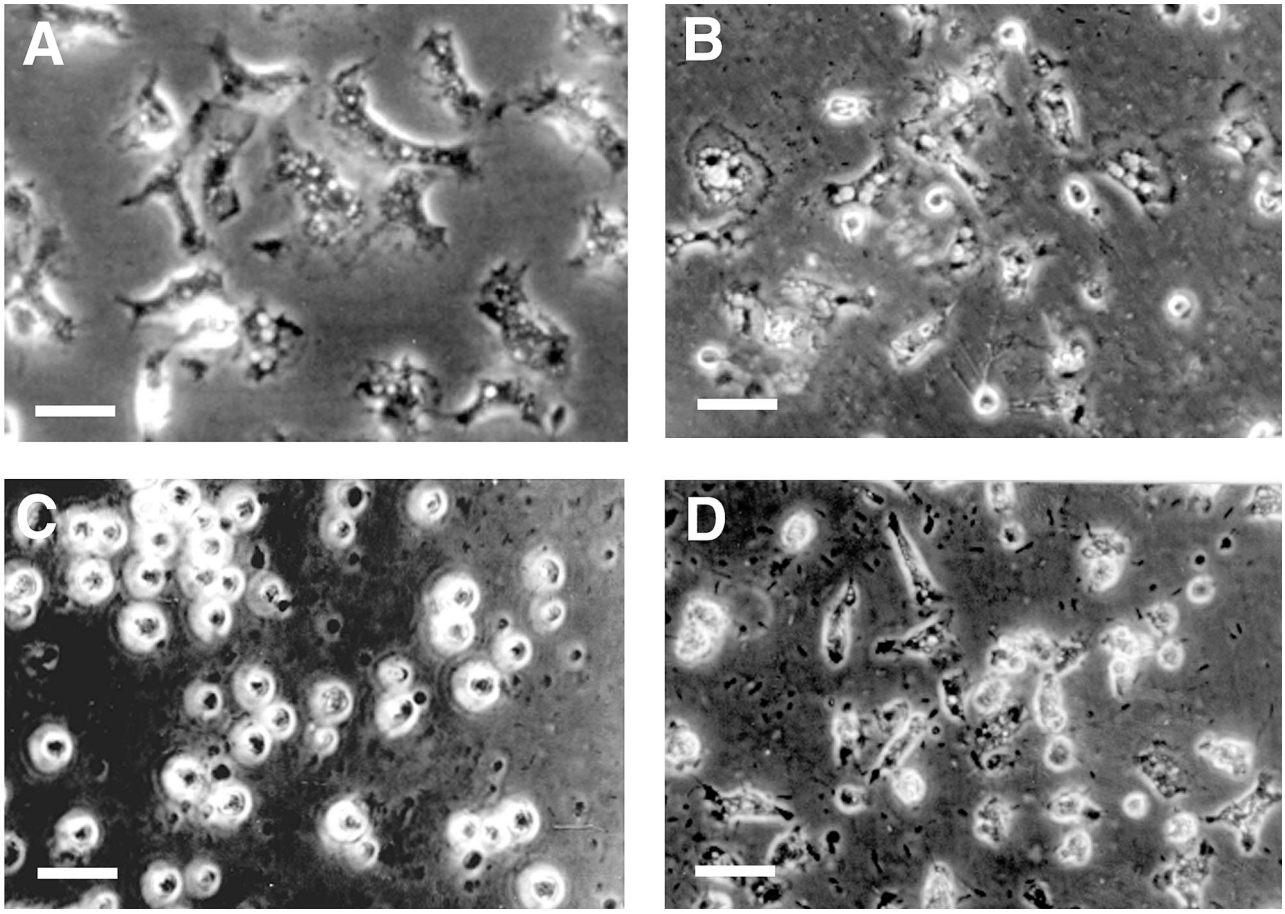

Figure 1. Rounding of *Mytilus edulis* haemocytes after exposure for 3 h at 20 °C to A) *Mytilus* haemolymph, B) 50 colony forming units (cfu) *Alteromonas* 1-1-1 per haemocyte suspended in *Mytilus* haemolymph, C) 50 cfu *Vibrio anguillarum* 2981 per haemocyte suspended in *Mytilus* haemolymph, and D) 50 cfu *V. anguillarum* 2981 per haemocyte suspended in filter-sterilized seawater. Scale bar = 20 μm.

3. RESULTS

3.1. Effect of bacterial strains on *Mytilus edulis* haemocytes

Mytilus edulis haemocytes adhered readily to plastic surfaces within 1 h at room temperature and their movement was recorded for 4 h by time-lapse video recording. A high proportion of cells remained spread on the surface for several hours (*figure 1a*) and displayed rapid, random sweeping movement across the plastic surface with occasional resting periods when cells remained rounded for several minutes before resuming active movement. When *Alteromonas* 1-1-1 was added, haemocytes appeared to be unaffected (*figure 1b*) for at least 3 h and continuous movement by the haemocytes was observed. On the other hand, when washed cell suspensions of *V. alginolyticus* 1339 or *V. anguillarum* 2981 (*figure 1c, d*) were added at ratios of 10 or 50 bacteria per haemocyte normal movement patterns ceased after approximately 120–150 min, by which time almost all haemocytes had become rounded; they did not resume normal movement within the next 16 h, the longest time of recording.

3.2. Kinetics of haemocyte rounding induced by *V. anguillarum*

For untreated haemocytes the percentage of cells which became rounded on the plastic surface gradually increased to 19 % over 3 h (*figure 2*).

On exposure to *V. anguillarum* 2981 at a bacteria/haemocyte ratio of 50:1 approximately 50 % of haemocytes were rounded by 90 min and almost all within 3 h (*figure 2*), the differences in rounding between bacteria-treated and untreated haemocytes being highly significant (Student's $t = 18.5$, 10.8 and 22.4 at 60, 120 and 180 min, respectively, 6 d.f., $P < 0.001$ in all cases). Formalin-treated cells of *V. anguillarum* 2981 did not cause cell rounding to occur within the same time scale.

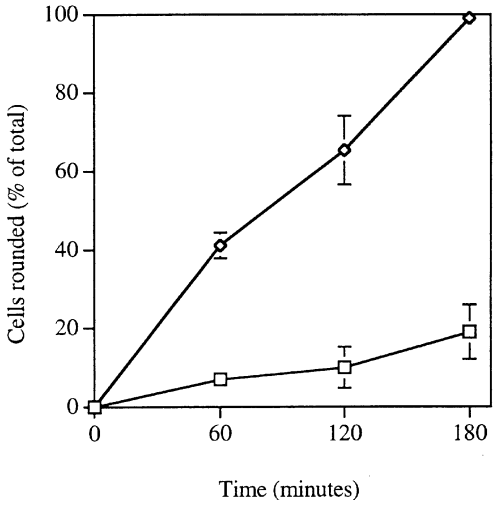

Figure 2. Kinetics of rounding of *Mytilus edulis* haemocytes induced by *Vibrio anguillarum* 2981. Washed bacterial cells suspended in filtered haemolymph were applied to monolayers of *M. edulis* haemocytes at bacteria/haemocyte ratios of 50:1. From photographs taken at various times the percentage of rounded cells was calculated, the results being normalized to take account of the fraction of cells rounded in samples at the beginning of the experiment. In four experiments the mean percentage of cells spread at the beginning of the experiment was 94 %. □, control cells; ◇, cells exposed to *V. anguillarum* 2981 cells. The mean values and standard deviations for results of four separate experiments are shown.

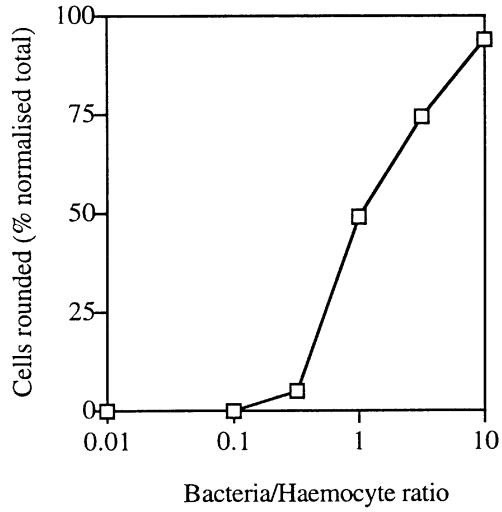

Figure 3. Rounding of haemocytes induced on incubation with various concentrations of *Vibrio anguillarum* 2981 for 3 h at 20 °C.

3.3. Response of haemocytes to different concentrations of *V. anguillarum*

The number of bacteria required to interact with a haemocyte to cause cell rounding was investigated using *V. anguillarum* 2981/haemocyte ratios over the range 0.1–10 and determining the percentage cells rounded after 150 min. When data were normalized to allow for the fraction of cells rounded in untreated control groups (*figure 3*) it appeared that approximately 50 % of attached haemocytes became rounded at a bacteria/haemocyte ratio of 1. Similar results were obtained for *V. anguillarum* strain A7, isolated from a recent episode of bivalve vibriosis in the UK.

3.4. Requirement for haemolymph

For the cell rounding effect to occur it was necessary to suspend bacteria in *M. edulis* haemolymph before addition to the haemocytes. If washed bacteria were suspended in sterile seawater no significant difference was observed between control and bacteria-treated haemocytes even at bacteria/haemocyte ratios of 500:1 for incubation periods of up to 4 h.

3.5. Nature of the haemolymph factor

The properties of the haemolymph factor were investigated briefly. At a dilution of 1/64 approximately 50 % of the haemolymph activity remained (*figure 4*). Approximately half of the haemolymph activity was destroyed on heating at 56 or 100 °C for 30 min, and the activity was lost after treatment of haemolymph with trypsin for 1 h at 25 °C.

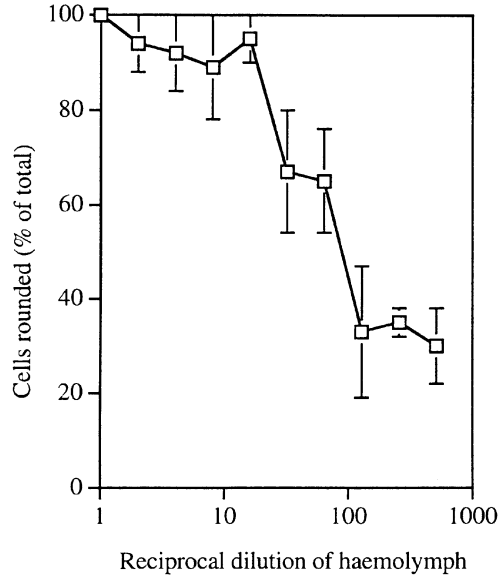

Figure 4. Effect of dilution on the ability of *Mytilus edulis* haemolymph to promote haemocyte rounding by *Vibrio anguillarum* 2981. Washed cells of *V. anguillarum* 2981 were suspended in serial dilutions of filtered haemolymph in filter-sterilized seawater and exposed to *M. edulis* haemocytes spread on the plastic surface of a 24-well microtitre plate. The proportion of cells rounded after incubation at room temperature for 2 h 30 min was determined. The mean values and standard deviations for results of three separate experiments are shown.

3.6. Toxicity of various bacteria to *M. edulis* haemocytes

Eight characterized marine vibrios and *Alteromonas* 1-1-1 were tested for their toxicity to haemocytes at bacteria/haemocyte ratios of 10:1 and 50:1. Two strains (*V. anguillarum* 2981 and *V. alginolyticus* 1339 were highly toxic, causing rounding of almost all cells at a ratio of 10 bacteria per haemocyte and four further strains (*V. anguillarum* strains 4579, 5679, B1 and NCMB6) showed medium toxicity, causing > 90 % cell rounding at a ratio of 50 bacteria per haemocyte, and between 34 and 66 % cell rounding at a ratio of 10 bacteria per haemocyte (*table I*). A total of 226 other bacteria, isolated from larvae, algae and water from oyster hatcheries and larvae, rotifers, algae and water from a turbot hatchery, have been tested for toxicity to haemocytes (*table II*). Thirteen of the 18 bacterial isolates which were highly toxic to haemocytes originated from the 52 isolates from a hatchery which experienced episodes of disease, indicating a highly significant correlation between isolate toxicity and disease in comparison with the total sample (13/18 compared to 52/226, $\chi^2 = 18.2$, $P < 0.001$ for 1 degree of freedom) or the non-disease isolates (13/52 compared with 5/174, $\chi^2 = 23.8$, $P < 0.001$ for 1 degree of freedom).

3.7. Effect of *V. anguillarum* 2981 culture supernatant on haemocytes

Bacteria-free culture supernatants of *V. anguillarum* 2981 were tested for their effects on mussel haemocytes. Neat culture supernatant induced rounding in 100 % of haemocytes and there was a dose-dependent response with 50 % cell rounding occurring at a dilution of approximately 1/500 (*figure 5*). Of 14 isolates from bivalve hatcheries for which the culture supernatants were tested at neat and 1/2 dilution, only one caused 100 % cell rounding when exposed to the 1/2 dilution and 8/14 induced no cell rounding even with neat culture supernatant.

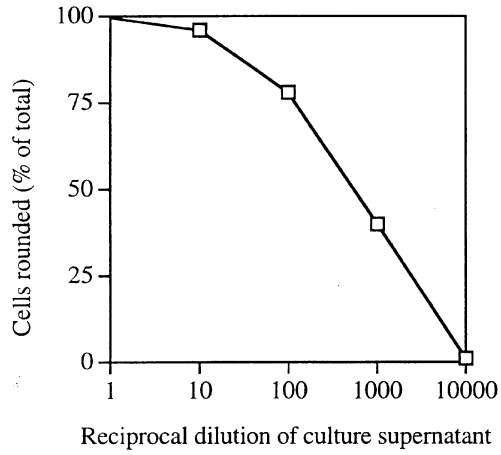

Figure 5. Toxicity of serial dilutions of bacteria-free culture supernatant of *Vibrio anguillarum* 2981 to haemocytes of *Mytilus edulis* after incubation for 3 h at 20 °C.

4. DISCUSSION

For vibriosis in bivalve hatcheries a number of possible mechanisms, such as the production of ciliostatic and lethal toxins, have been recognized [26]. However, these toxin-mediated mechanisms explain only one of the three broad classes of disease described [9]. Bacterial interactions with haemocytes are inevitable during invasive infections or when bacteria are ingested during the normal filtration and feeding processes. In microbial infections the interaction between micro-organisms and phagocytic cells is a crucial determinant in the disease process.

The requirement for haemolymph to facilitate interaction of bacteria with haemocytes is characteristic of an opsonin. A calcium-dependent opsonin of *M. edulis* has been purified [28] and a cell-surface location on haemocytes was suggested from work with antiserum to the purified opsonin. From *C. gigas* two haemagglutinins, gigalins E and H, have been described [27]. These were part of large molecular weight complexes in the haemolymph and opsonic activity towards *V. anguillarum* NCMB6 was demonstrated in vivo [14]. More recently, a haemagglutinin/lectin has been purified from the horse mussel, *Modiolus modiolus* which bound to a range of marine bacteria [32]. In the current assay, the opsonic factor in haemolymph may be similar to these factors [29, 32] but cross-absorption of haemolymph with human erythrocytes or yeast cells was not performed. Since approximately half the haemolymph activity was lost on heating both at 56 °C and at 100 °C, two or more different active factors may be involved. However, the sensitivity of the haemolymph activity to inactivation by trypsin suggests that it is protein or peptide in nature.

A second possibility is that, rather than acting as an opsonin, the haemolymph acted as a growth medium enabling bacteria to multiply to a much higher level in haemolymph than in seawater within the 150 min normally employed in the assay. Although multiplication of bacteria in haemolymph was evident over periods of a few hours, several factors suggest that this does not explain the requirement for haemolymph. First, bacteria/haemocyte ratios of 500:1 in seawater had only a slight effect on haemocytes, whereas rounding of haemocytes could occur within 150 min with bacteria/haemocyte ratios of 10:1 when suspended in filtered haemolymph. Growth in haemolymph is unlikely to be so rapid. Second, no difference was observed in the fraction of cells rounded at 10 and 20 °C (results not shown), whereas a much higher growth rate would be expected at the higher temperature. Third, heating the haemolymph at 56 °C for 30 min halved the activity, and it is unlikely

that substrates for bacterial growth would be inactivated under such conditions.

Rounding of haemocytes can be taken to indicate a cessation of phagocytic activity since pseudopodium formation is an essential part of phagocytic activity [29, 30]. The observed effect occurred relatively slowly, requiring approximately 90 min for 50 % of cells to become rounded. The dose–response curve (*figure 3*) indicated that very few bacteria were required per haemocyte to cause cell rounding. The true number was not established because some replication of bacteria would occur during the incubation period. Also, the bacteria/haemocyte ratio used was based on the total haemocyte concentration in the haemolymph and not all these haemocytes adhered to the surface of the tissue culture assay plate.

The mechanism whereby bacteria caused cell rounding and inhibited further phagocytosis was not determined. Such inhibition could occur via interaction of a surface component of the bacterium or by a product excreted by the bacterium after phagocytosis. The finding that formaldehyde-killed bacteria had lost the ability to induce cell rounding indicates that bacterial cell viability is important, possibly to allow export of a bacterial component. However, it may be that the active site of a surface molecule is inactivated by this treatment. Sterile bacterial culture supernatants of *V. anguillarum* 2981 also caused cell rounding but it is not possible, from the currently available data, to differentiate between a secreted bacterial product and a cell-surface component as the latter are often released in association with lipopolysaccharide vesicles [13]. Toxins active against the eukaryotic cytoskeleton have been described for several bacteria. These include the ADP-ribosylating toxins secreted by several clostridia [1] which cause disruption of the cytoskeleton by modification of G-actin leading to depolymerization of F-actin, a crucial component of the cytoskeleton. Also, the cytotoxic necrotizing factor (CNF-1) of certain strains of *Escherichia coli* induces reorganisation of the actin cytoskeleton into 'stress fibres' and also induces 'invasion' of cells [11]. The mechanism of induction of the cell-rounding process is, as yet, unclear, but is unlikely to involve the above types of toxins. However, it is possible that either bacteria or a secreted toxin could induce apoptosis, as has been shown to occur when macrophages encounter *Shigella flexneri* cells or *Bordetella pertussis* adenylate cyclase toxin [17, 35].

It was shown recently [18] that a range of bacteria pathogenic for bivalve larvae induced an inhibition of chemiluminescence activity in adult bivalve haemocytes when exposed to specific pathogens. The intensity of the response was not correlated with the virulence of the bacteria; however, haemocytes preexposed to bacteria varied in their subsequent chemiluminescence response to zymosan, and this was correlated with bacterial virulence. As the chemiluminescence response of haemocytes results from membrane interaction with bacteria it is possible that the events reported in this study and by Lambert and Nicolas [18] are related.

Ingestion of bacteria by bivalves normally results in degradation of the bacteria by a mechanism involving lysozyme of the digestive tract of the bivalve [3]. Lysozyme may not be present in significant quantities in the early stages of larval development and this, coupled with the toxicity of certain groups of bacteria to haemocytes, may be related to the high susceptibility of larvae to bacterial infection. The rapid inhibition of the filtration response of mussels by washed suspensions of vibrios [19] may be connected with the present findings, although further investigation of the bacteria/haemocyte interaction is required to define the mechanisms involved. Such studies are currently in progress.

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