APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1978, p. 169-177
0099-2240/78/0036-0169$02.00/0

Vol. 36, No. 1
Printed in U.S.A.

Dependence of Marine Bdellovibrios on Potassium, Calcium, and Magnesium Ions

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Received for publication 7 February 1978

Marine bdellovibrios show a specific requirement for K^+, Ca^{2+}, and Mg^{2+}. Potassium is essential for high velocity and seems to be necessary for attachment of the free bdellovibrios. Calcium and magnesium are necessary for attachment and penetration. Magnesium also plays a role in maintaining the integrity of the bdelloplast. The adaptation of these bdellovibrios to the marine environment is manifested by their stringent cation requirements.

Marine bdellovibrios which attach to many different types of gram-negative bacteria have recently been isolated from various marine environments (2, 6, 13, 14, 17-19, 22, 23, 25). These bdellovibrios are similar to the previously described terrestrial and freshwater types in their biphasic life cycle, which includes obligate alternation between a free-living motile form and an aflagellate one that multiplies within the periplasmic space of potential host cells (17), and in their division by multiple fission rather than by the binary fission typical of most procaryotes. However, the marine bdellovibrios differ from other bdellovibrios in their requirement for high concentrations of salt and in their requirement for the four major cations present in seawater: Na^+, K^+, Mg^{2+}, and Ca^{2+}. They also differ in the low guanosine-cytosine ratio of their DNA (17, 25). In the present paper, the qualitative and quantitative nature of the cation requirements of selected marine bdellovibrios strains are described. It is shown that the different developmental stages require different cations and that there is a complex interrelation between the various cations.

MATERIALS AND METHODS

Bacterial strains. *Photobacterium leiognathi* LR-101, isolated from the Gulf of Elat, served as the host organism. Bdellovibrio strains BM1, -2, -3, -4, -5, -6, -11, -12, -14, and -15, all isolates from the Mediterranean, have been previously described (17). Maintenance of all the above strains, as well as determination of the plaque-forming capacity of the bdellovibrio strains, was as described previously (17).

Media. The following four basic salt solutions were used for preparation of media and buffer: (i) synthetic marine salt solution (SM) containing: NaCl, 500 mM; KCl, 10 mM; $MgSO_4$, 25 mM; $MgCl_2$, 25 mM; and $CaCl_2$, 10 mM; (ii) complete salt solution (CS) containing: NaCl, 500 mM; and KCl, $MgCl_2$, and $CaCl_2$, 10 mM each; (iii) low-concentration complete salt solution (LS) containing: NaCl, 500 mM; KCl, 0.5 mM; $MgCl_2$, 0.5 mM; and $CaCl_2$, 5 mM; and (iv) sodium chloride base solution (NaB) containing 500 mM NaCl. Tris(hydroxymethyl)aminomethane-buffered salt solutions NaBT, CST, LST, and SMT were prepared by adding a 10 mM final concentration of tris(hydroxymethyl)aminomethane (Fluka) to the appropriate base solution and adjusting the pH to 7.5 with HCl.

Rich media NaBPY and SMPY were prepared by adding 0.5% peptone (P) (Difco) and 0.3% yeast extract (Y) (Difco) to the appropriate basic salt solutions. Diluted media LS (PY/10) and SM(PY/10) were prepared by adding a one-tenth concentration of the nutrients. In both cases, the pH was adjusted to 7.5 with NaOH. Solid media were prepared with Oxoid agar no. 1, using a 0.5% concentration for the bottom layer and 0.4% for the top layer.

The standard double agar layer technique was used to test the cation requirement for plaque formation. The complete salt solution in 10 mM tris(hydroxymethyl)aminomethane buffer contained 500 mM NaCl, 50 mM $MgCl_2$, and 10 mM each of $CaCl_2$ and KCl, minus the cation tested, which was added at various concentrations. The host bacterium was inoculated into the top layer at a concentration of 2.5×10^{10} cells per ml, resulting in a lawn sufficiently confluent and opaque to allow growth of bdellovibrio plaques and their discernment. The addition of nutrients for host growth was eliminated by this technique. Plaque-forming efficiency in the complete salt solution was determined as 1.0.

Cultures of bdellovibrios for the different salt composition experiments were grown on host LR-101 in LST buffer. The salt concentration was then reduced by dialyzing 6-ml bdellovibrio samples for 3 h against 500 ml of NaBT buffer changed hourly (Grove Englander Micro Dialyzer, MD 102, Hoefer Scientific Instruments, Inc.; dialysis tubing no. 8 [0.25 inch; ca. 6.4 mm], Will Scientific, Inc.). The host for these experiments was grown in NaBPY medium, washed twice, and resuspended in NaBT buffer. The infection systems contained the dialyzed bdellovibrios and host

cells (5×10^8 and 1×10^9 cells per ml, respectively) suspended in NaBT to which concentrated salt solutions of KCl, $CaCl_2$, and $MgCl_2$ were added to a 10 mM final concentration of each. The K^+, Ca^{2+} and Mg^{2+} impurities present in an NaBT infection system (0.04 mM K, 0.02 mM Mg, 0.09 mM Ca) were below those required for bdellovibrio growth (Table 1, strain BM4). A similar concentration of bdellovibrios without host was used for assessing velocity. All the experiments described in this paper were carried out at 25°C.

Radioactive labeling. Radioactively labeled bdellovibrios were obtained by adding 1 μCi of L-[*U*-^{14}C]-leucine (Amersham; specific activity, 330 mCi/mmol) per ml to a growing bdellovibrios culture.

Measurement of attachment and penetration. The kinetics of attachment and penetration were measured using radioactive bdellovibrios according to Varon and Shilo (26), but with several modifications necessitated by the marine characteristics of the organisms used and the smaller dimensions of the host.

For attachment kinetics, 100-μl aliquots were added to test tubes containing 5 ml of washing solution and 0.05 ml of concentrated formaldehyde as a fixative.

TABLE 1. *Cation requirement for marine bdellovibrio strains BM4 and BM11*[a]

Cation	Salt added (mM)	Length of incubation until plaque appearance (days)		Plaque-forming efficiency	
		BM4	BM11	BM4	BM11
K^+	KCl				
	0	–	–	$<10^{-4}$	$<10^{-4}$
	0.5	–	–	$<10^{-4}$	$<10^{-4}$
	0.1	3	–	0.5	$<10^{-4}$
	0.5	2	2	1.1	0.7
	1	2	1	1.0	0.9
	5	1	1	1.1	1.06
	10	1	1	1.0	1.00
Mg^{2+}	$MgCl_2$				
	0	–	–	$<10^{-4}$	$<10^{-4}$
	0.05	2	–	0.26	$<10^{-4}$
	0.5	1	–	1.1	$<10^{-4}$
	5	1	3	1.08	1.2
	10		2		1.3
	25		2		1.2
	50	1	1	1.0	1.0
Ca^{2+}	$CaCl_2$				
	0	–	–	$<10^{-4}$	$<10^{-4}$
	0.05	–	–	$<10^{-4}$	$<10^{-4}$
	0.1	–	–	$<10^{-4}$	$<10^{-4}$
	0.5	–	–	$<10^{-4}$	$<10^{-4}$
	1	1	–	0.88	$<10^{-4}$
	5	1	1	0.94	0.8
	10	1	1	1.0	1.0

[a] Plaque-forming capacity was tested by the standard double agar layer technique. –, No plaque appearance within 6 days of incubation. The complete salt solution in 10 mM tris(hydroxymethyl)aminomethane buffer contained 500 mM NaCl, 50 mM $MgCl_2$, and 10 mM each $CaCl_2$ and KCl.

The contents were then filtered through membrane filters (pore size, 0.8 μm) and washed with 100 ml of washing solution. The washing solution was essentially that of Varon and Shilo (26), but also contained 500 mM NaCl and 10 mM concentrations of KCl, $CaCl_2$, and $MgCl_2$. Samples for the determination of total radioactivity were filtered through membrane filters (pore size, 0.22 μm). Filters were deposited in scintillation vials. After the filters dried overnight at 35°C, 10 ml of scintillation fluid (3 g of PPO [2,5-diphenyloxazole] and 0.3 g of POPOP [1,4-bis(2-C-4-methyl-5-phenyloxazolyl)benzene] [Packard] per liter of toluene) was added to each vial, and the radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrophotometer, model 3380. The radioactive data obtained do not differentiate between penetration and attachment.

Penetration per se was determined similarly in parallel to the attachment measurements, but the samples were first mechanically agitated with the aid of a Mini Omnimixer.

Velocity measurements. The bdellovibrio cultures were photographed within the first 10 min of addition of a particular salt solution via the microscope by television camera (Sony AVC 1400 CE), and their velocity path was recorded on video tape (International Video Corp., model IVC 871P). Frame-by-frame analysis (time base, 50 frames per s) was carried out with the aid of a television monitor (G. P. L. Singer Video Center 5-inch monitor); the path of individual motile cells was traced, and the number of frames forming the path was counted. The length of the path was measured, and, using a micrometer stage as the reference, the velocity was expressed in micrometers per second.

Cation concentration measurement. The concentrations of K^+, Ca^{2+}, and Mg^{2+} were measured with the aid of a Perkin-Elmer atomic absorption spectrophotometer (model 403), using air-acetylene, at 766.5, 422.7, and 285.6 nm, respectively. CsCl was added for the K^+ readings and $LaCl_3$ was added for the Ca^{2+} and Mg^{2+} readings (20) to prevent suppression.

Phase microscopy photography. A Zeiss Universal microscope with phase attachment was used for microscopy and microphotography.

RESULTS

Cation requirement for growth and plaque formation. Whereas all the Mediterranean bdellovibrio strains isolated by us grow well in appropriate two-membered cultures in seawater and buffered marine salt solution (liquid as well as solid medium), none of the strains is able to multiply in buffered NaCl solution without the addition of Ca^{2+}, Mg^{2+}, and K^+.

The requirement for each of the different cations present in SMT was quantitatively determined. Table 1 shows the cation requirement for plaque formation on *P. leiognathi*, the host for two selected bdellovibrio strains (BM4 and BM11). Both strains required the same salt composition, i.e., the addition of K^+, Ca^{2+}, and Mg^{2+} to the NaBT buffer, though in different concen-

trations. In general, the salt requirement for the above three cations was significantly higher for BM4 than for BM11. Based on plaque-forming efficiency, this difference was 5-fold in the case of KCl and $CaCl_2$ and 100-fold for $MgCl_2$. The effect of NaCl concentration was similar within the range between 400 and 500 mM, indicating that the effect of KCl, $CaCl_2$, and $MgCl_2$ was specific and not osmotic for both strains.

The anion moiety of the salts used, except possibly Cl^-, does not seem to be important for bdellovibrio development; exchange of $MgSO_4$ in the growth medium by $MgCl_2$ did not affect growth.

Effect of K^+, Mg^{2+}, and Ca^{2+} on the motility of bdellovibrios and their interaction with host cells. One of the remarkable characteristics of bdellovibrio cells is their rapid movement, an indispensible requirement for stable attachment to the host (26). Table 2 shows the effect of different cations on the velocity of BM4 cells used in this and the experiments following. It can be seen that the absence of K^+ markedly reduced the velocity (fourfold), whereas the addition or removal of Ca^{2+} and Mg^{2+} did not significantly alter it ($P > 0.6$ for media lacking K^+ with or without Ca^{2+} and Mg^{2+}, and $P > 0.8$ for media containing K^+ with or without Ca^{2+} and Mg^{2+}). Therefore, rapid motility depended on the presence of K^+ and not on the other cations tested. In addition, potassium increased the number of cells that were motile; 50% of the population was motile in its presence, compared to only about 20% in its absence.

Effect of the cation composition of the medium on the different stages of the bdellovibrio life cycle. Phase microscopy of bdellovibrio growth under optimal laboratory conditions (CST buffer) revealed the attachment of many cells already during the first few minutes after the host and bdellovibrio cells were mixed, and the appearance of bdelloplast aggregates typical for this system within 15 to 30 min. Within 4 h, the intraperiplasmic cycle was completed, and progeny cells were released which immediately attached to the residual host cells, again forming bdelloplast aggregates (Fig. 1).

TABLE 2. *Average velocity of marine bdellovibrio strain BM4 in different salt solutions*

Salt composition[a]	Avg velocity (μm/s)
NaCl	21.8 ± 1.5
NaCl, $CaCl_2$, $MgCl_2$	21.1 ± 1.5
NaCl, KCl	86.4 ± 9.9
NaCl, KCl, $CaCl_2$, $MgCl_2$	89.1 ± 5.7

[a] NaCl concentration was 500 mM; all other salts were added at concentrations of 10 mM each. $n = 10$.

After 8 h, there was total lysis of the host cells, and only bdellovibrios remained in the culture. At the same time, there was a fourfold increase in plaque-forming units (PFU) (Fig. 2).

Attachment kinetics are shown in Fig. 3. Rapid attachment, most of it occurring within the first 10 min, was typical under such conditions. It was followed by rapid penetration, as shown in Fig. 4. In buffered salt solution containing only NaCl and KCl, no attachment occurred as measured kinetically with radioactively labeled bdellovibrios (Fig. 3) or as revealed microscopically; neither bdelloplasts nor lysis was detected even after 48 h.

The first growth cycle in buffer containing Na^+, K^+, and Mg^{2+} but lacking $CaCl_2$ resembled that in complete buffer (CST): attachment during the first few minutes though with a somewhat lower percentage of attachment (Fig. 3), the appearance of bdelloplast aggregates within 15 to 30 min, and the release of progeny cells within 4 h. The number of bdellovibrio cells (PFU) increased during the first 4 h (Fig. 2). However, after completion of the first cycle, there was no further infection, though uninfected host cells and highly motile bdellovibrios were still present in the culture. Even after 24 and 48 h, no additional infection was apparent. It seems that in the absence of added Ca^{2+} in the infection system, the parasite is able to complete one cycle only, and the daughter cells then released cannot initiate a new infection.

The lack of additional cycles in the absence of added Ca^{2+} could be due to changes undergone by some or all of the host cells or the bdellovibrios or to changes in the medium. To examine these possibilities, the following experiments were done: (i) residual host cells after termination of the single growth cycle were added to fresh bdellovibrio cells in a fresh Ca^{2+}-deficient medium; (ii) fresh bdellovibrios and fresh host cells were suspended in the spent medium after completion of the initial single cycle. In both cases, the fresh bdellovibrios completed one growth cycle and did not initiate a second one. This led to the assumption that the inability to undergo an additional single cycle in Ca^{2+}-deficient medium was due to a change in the bdellovibrio cells themselves. This assumption was checked by examining bdellovibrios released at the end of the growth cycle. It was found that these bdellovibrio progeny were unable to initiate a second growth cycle even after the medium or host or both were substituted by fresh medium and/or host cells.

Addition of $CaCl_2$ after termination of the first cycle (6 h [Fig. 5] or even later [not shown]) evoked the immediate renewal of attachment and additional cycles of intraperiplasmic growth,

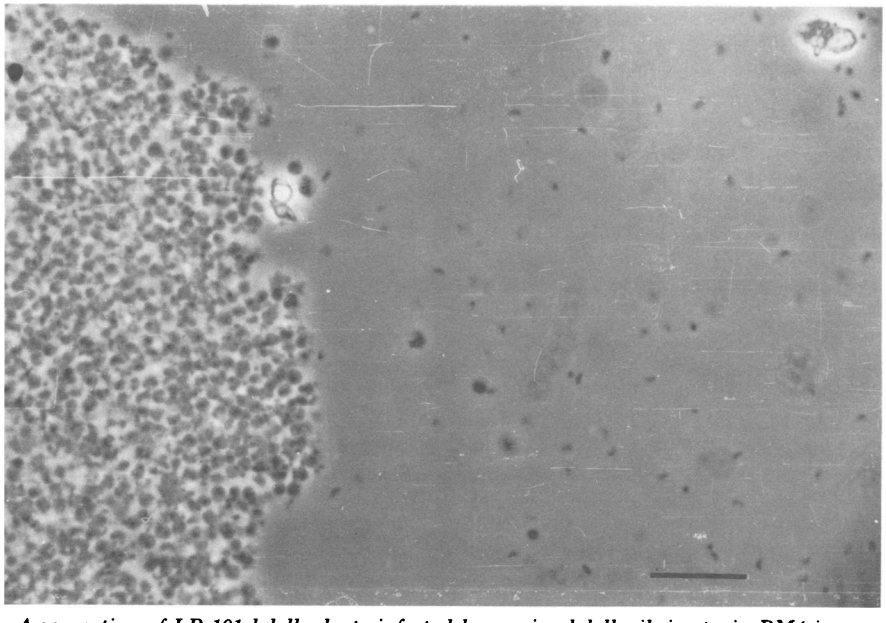

FIG. 1. *Aggregation of LR-101 bdelloplasts infected by marine bdellovibrio strain BM4 in complete salt solution (bar = 10 μm).*

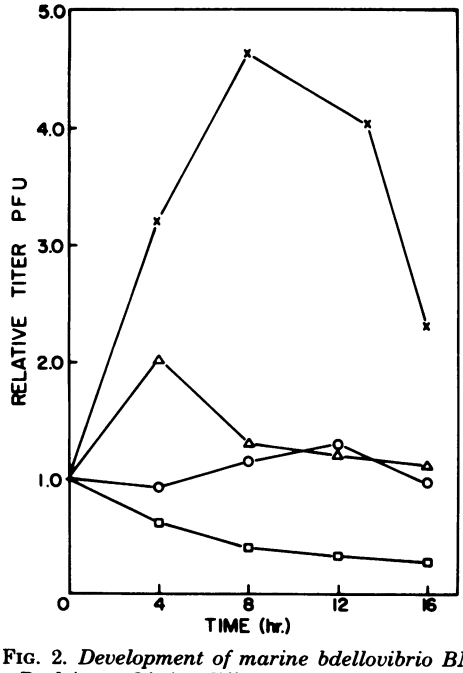

FIG. 2. *Development of marine bdellovibrio BM4 on P. leiognathi in different salt solutions. All buffered solutions contained 500 mM NaCl and 10 mM each of the salts specified: $MgCl_2$ and $CaCl_2$ (□); KCl and $CaCl_2$ (○); KCl and $MgCl_2$ (△); KCl, $MgCl_2$, and $CaCl_2$ (×). PFU on SM(PY/10) medium. Initial concentrations of bdellovibrio and host cells were 5 × 10^8 and 1 × 10^9 cells per ml, respectively.*

accompanied by bursts of bdellovibrio progeny (Fig. 6). The decrease in PFU observed after Ca^{2+} addition most likely reflects the clumping and aggregation of the bdelloplasts, a phenomenon observed microscopically and also discerned during the initial first cycle prior to the cation's addition.

In buffer containing Na^+, K^+, and Ca^{2+} but lacking Mg^{2+}, microscopic observations revealed that the bdellovibrio-host interactions were less

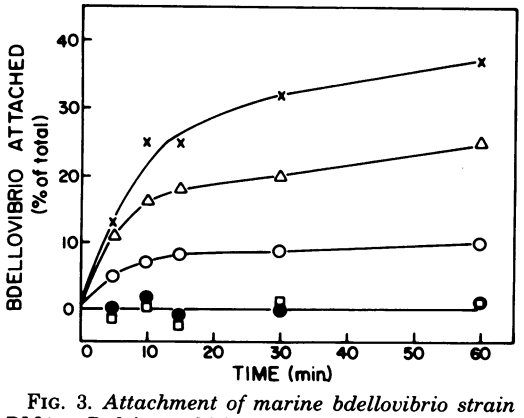

FIG. 3. *Attachment of marine bdellovibrio strain BM4 to P. leiognathi in different salt solutions. All buffered solutions contained 500 mM NaCl and 10 mM each of the salts specified: KCl (●); $MgCl_2$ and $CaCl_2$ (□); KCl and $CaCl_2$ (○); KCl and $MgCl_2$ (△); KCl, $MgCl_2$, and $CaCl_2$ (×).*

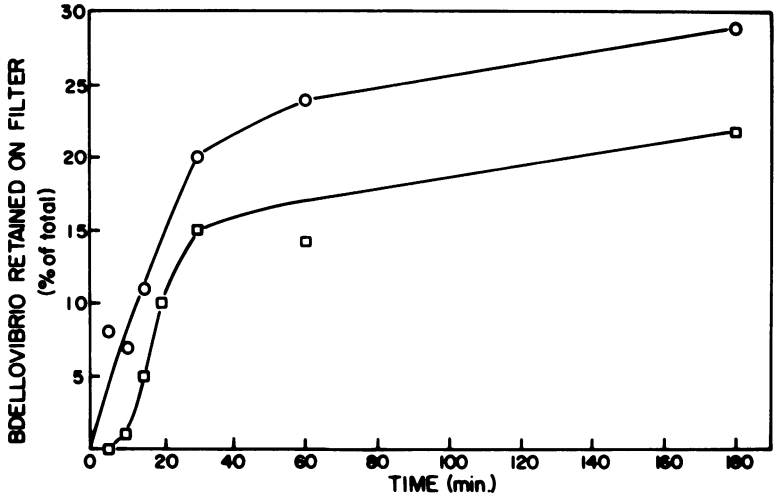

FIG. 4. *Attachment (○) and penetration (□) of marine bdellovibrio strain BM4 to P. leiognathi in buffered complete salt solution.*

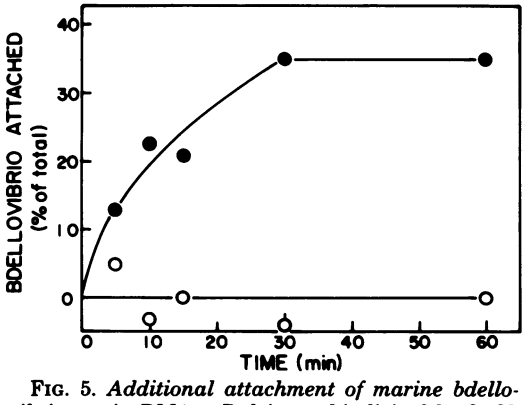

FIG. 5. *Additional attachment of marine bdellovibrio strain BM4 to P. leiognathi elicited by $CaCl_2$ addition. An infection system, suspended in salt solution containing 500 mM NaCl and 10 mM each of KCl and $MgCl_2$, was subdivided into two after completion of the first infection cycle, and the $CaCl_2$ was added (final concentration, 10 mM) to one of the samples. Additional attachment was then measured. (●) System with additional $CaCl_2$; (○) system without added $CaCl_2$.*

stable than those in the complete buffer. The kinetics of attachment and penetration were slower than in the systems described above (Fig. 3, 7a, and 8). Bdelloplasts were observed microscopically after 3 to 4 h, compared to 15 to 30 min in complete buffer. In addition, after 6 to 8 h, free extracellular spirals appeared, a number of which seemed attached to the host cells (Fig. 7b). During the next 3 h, the number of spirals within the microscopic field increased, subsequently decreasing. Their decrease was accompanied by the appearance of increased numbers of "normal-sized" bdellovibrio cells and larger numbers of PFU (Fig. 2). During this period, empty host cells were seen, and the infection

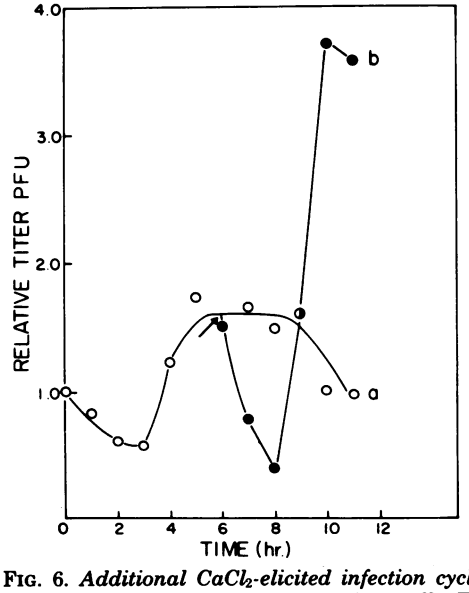

FIG. 6. *Additional $CaCl_2$-elicited infection cycles of marine bdellovibrio strain BM4 on host cells. The infection system, suspended in salt solution containing 500 mM NaCl and 10 mM each KCl and $MgCl_2$, was subdivided into two after 6 h of incubation. $CaCl_2$ was added to a final concentration of 10 mM (arrow) to one of the cultures. (a) Development of one infection cycle in the original system and the lack of additional cycles in the subdivided one lacking $CaCl_2$ (○); (b) development of additional cycles in the subdivided system with added $CaCl_2$ (●). PFU on SM(PY/10) medium.*

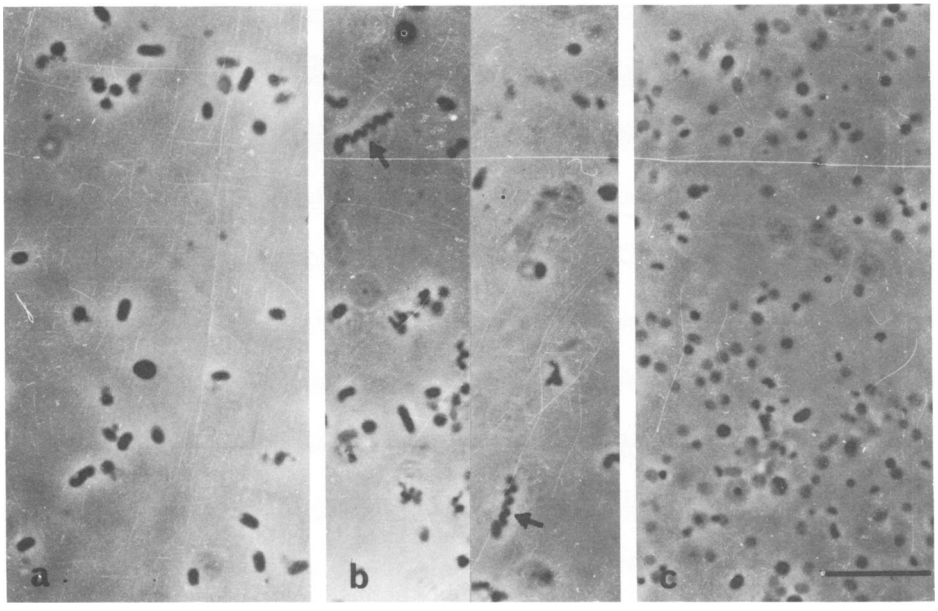

FIG. 7. *Stages in the infection cycle of bdellovibrio strain BM4 in Mg^{2+}-deficient CST: (a) 15-min incubation, attachment to host LR-101; (b) 9.5 h, spirals of bdellovibrios (indicated by arrows); (c) 24 h, empty host LR-101 cells and bdellovibrio cells (bar = 10 μm).*

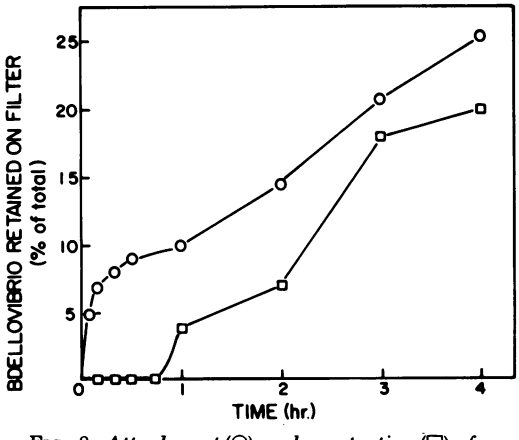

FIG. 8. *Attachment (○) and penetration (□) of marine bdellovibrio strain BM4 to host cells in Mg^{2+}-deficient CST.*

terminated when the culture contained only free bdellovibrios and empty host cells (Fig. 7c). The appearance of extracellular spirals prior to their multiple fission seems to be associated with premature bdelloplast lysis. This is borne out by the fact that, close to the time of free spiral appearance, spirals could still be observed intraperiplasmically (Fig. 9). Free bdellovibrios did not develop into spirals in the presence of filtrates of medium in which extracellular spirals had already appeared. According to microscopic observations, neither did bdellovibrios attached to host cells appear to lengthen into extracellular spirals. The fact that the extracellular spirals subsequently disappeared and concomitantly small-sized bdellovibrio cells increased in number indicates that fission of bdellovibrio cells had occurred extracellularly. Ca^{2+} could not substitute for Mg^{2+}; even a fivefold increase of the Ca concentration (50 mM) did not alter the sequence of events.

In buffer containing Na^+, Ca^{2+}, and Mg^{2+} but lacking K^+, the cells were of slow motility and there was no measurable attachment in experiments using radioactively labeled bdellovibrios (Fig. 3). Microscopic observations throughout 16 h confirmed this finding; even after 48 h, host cells remained unlysed. The number of PFU gradually decreased during incubation in the K^+-deficient medium (Fig. 2).

In the presence of NaCl alone or in combination with either $MgCl_2$ or $CaCl_2$, the cells were of slow motility and, as could be expected, the bdellovibrios under these conditions did not attach to their host cells.

DISCUSSION

The present results verify and extend our previous findings that marine bdellovibrios have a specific requirement for Na^+, K^+, Mg^{2+}, and Ca^{2+}, exceeding the exigence of high osmolarity, and need all the above four cations for growth

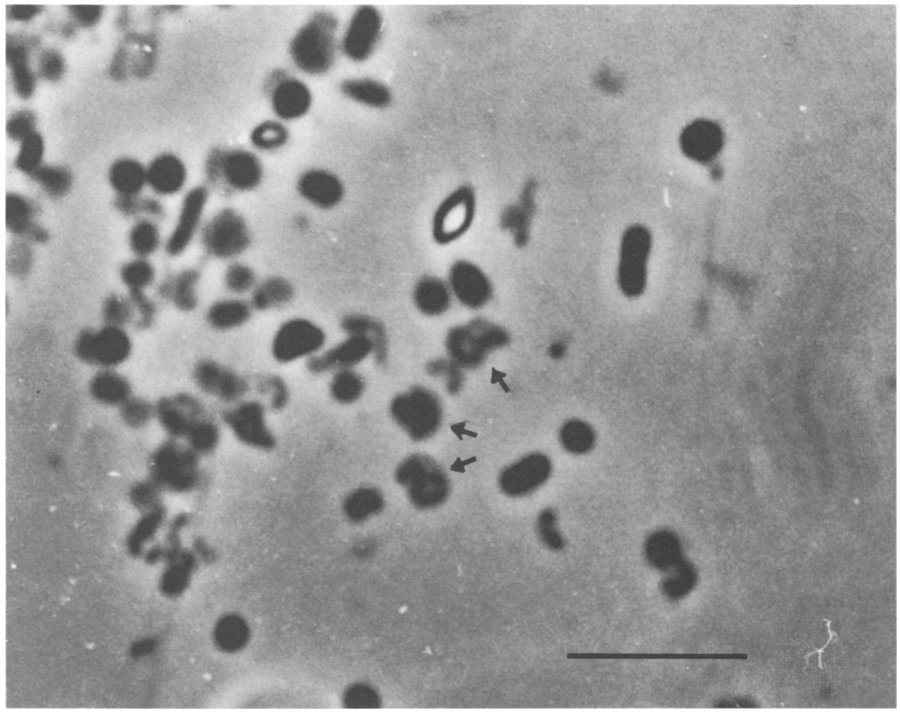

FIG. 9. *Bdelloplasts of host LR-101, including spirals of bdellovibrio strain BM4 (arrows), in Mg^{2+} deficient CST (8 h of incubation; bar = 5 μm).*

and development (17). In this respect, they can be considered true marine bacteria as defined by MacLeod (16). The cationic requirements manifested by the strains isolated from the Mediterranean are also shared by other marine bdellovibrios (2, 19, 25).

In contrast to the general effect of cations on infection by nonmarine strains, the specificity of the cation effect in the marine infection system and the need for a combination of defined cations emphasize the complex dependence of the marine bdellovibrios on their environment. The specific adaptation of the marine bdellovibrios to the constant ionic seawater composition clearly points out their indigenous nature in this habitat. The possibility, often raised, that all aquatic bdellovibrio strains originate in sewage (12) or are swept-in terrestrial types can thus be excluded.

The results of our experiments show that each of the different cations plays a specific role in the infection cycle of the bdellovibrio strain tested. It was found that potassium ions affected bdellovibrio velocity, although their effect on other yet unknown parameters cannot be excluded. Direct measurement of attachment kinetics as well as microscopic observations showed that in K^+-deficient media bdellovibrios were also incapable of attachment. The correlation between motility and attachment (1, 26) and the normal life cycle's dependence on motility have been suggested by earlier experiments (9, 24). The results of our work with potassium more directly advocate the necessity for motility of high velocity for attachment.

However, motility even of high velocity is not sufficient to ensure attachment. Repeated cycles of attachment demand the presence of Ca^{2+}, in the absence of which only a single cycle is completed. This might be attributable to traces of Ca^{2+} and/or a substance that requires Ca for its synthesis and is retained by the bdellovibrio cells. Magnesium stimulates attachment, penetration, and bdelloplast formation and seems to be essential for stabilization of the bdelloplast wall. In its absence, long bdellovibrio spirals were released into the medium, indicating "premature" bdelloplast lysis, followed by extraperiplasmic, multiple fission of the spirals. Both phenomena are similar to those observed in bdellovibrio-infected *Acinetobacter* (J. Castro e Melo, Ph.D. thesis, University of Pittsburgh, Pittsburgh, Pa., 1975). Many of the known marine bacteria require Mg^{2+} for maintenance of structural integrity (3, 7, 8, 11). Their bdelloplasts may be, therefore, especially sensitive to

disintegration under conditions of Mg^{2+} deficiency.

Experiments testing the effect of Na^+, K^+, and other monovalent cations on attachment in the absence of divalent cations have shown that monovalent cations are necessary and suffice for attachment of nonmarine bdellovibrio strain 109D and also that the different cations are interchangeable (Castro e Melo, Ph.D. thesis). In nonmarine bdellovibrio strain 6-5-S, the presence of monovalent cations enabled the completion of a full life cycle. In this case too, the requirement was nonspecific (4). In a Mediterranean strain (BM4), on the other hand, K^+ had a different effect than did Na^+; the presence of both was required in the medium, and they were not interchangeable (17). However, in the marine strains, the necessary monovalent cations did not suffice for attachment to take place.

A mechanism explaining the role of cations in attachment has been proposed by Castro e Melo, who suggested that, in the nonmarine strains, the fact that the different cations have a similar effect on attachment indicated that the host-bdellovibrio interaction begins as an electrostatic contact, rather than as any physiological interaction between the two. In infection systems with marine strain BM4, there was no attachment in media unless the media contained Na^+, K^+, and Ca^{2+}. Thus, at least in this case, the hypothesis that electrostatic forces are operative in the initial contact is invalid.

In their requirement for divalent cations, the marine strains also markedly differ from the nonmarine ones. In the nonmarine strains, the addition of divalent cations suffices to permit their complete development, and there are no other cation prerequisites (4, 5, 15, 21; Castro e Melo, Ph.D. thesis). Experiments with Ca^{2+}, Mg^{2+}, and other divalent cations revealed that these all had a similar effect (4; Castro e Melo, Ph.D. thesis) and were interchangeable. The presence of either Ca^{2+} (5, 21) or Mg^{2+} (21) gave results similar to a combination of the two.

The marine bdellovibrio types may be clearly differentiated from all known nonmarine ones by the stringent and specific concomitant requirement for several different cations as well as the large concentrations necessary for attachment and growth. This may be of special importance when dealing with bdellovibrios in ecosystems in which marine and nonmarine strains coexist, as in estuaries or sewage and in terrestrially polluted oceanic coastal waters (6, 10, 14).

The unique marine characteristics of the Mediterranean bdellovibrio strains studied, expressed in their cation requirements, are probably shared by other marine bdellovibrios. It should be stressed, however, that though many marine bdellovibrio strains show complex cationic requirements, the present detailed results for all four cations and their specific role in the developmental cycle are based on the study of a single strain only. It would be important to test the general nature of these cationic requirements for other marine bdellovibrio strains.

ACKNOWLEDGMENTS

We thank Mazal Varon for her critical reading of this paper. This study was supported by a grant from the Deutsche Forschungsgemeinschaft.

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