Virus-Like Particles in Microbial Population Control and Horizontal Gene Transfer in the Aquatic Environment

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ABSTRACT

Viruses, or Virus-Like Particles (VLPs), occur in large numbers (~10^5-10^8 VLPs/ml) and are acknowledged to be general constituents of aquatic ecosystems. The bactericidal effect of VLPs derived from cultured marine strains and natural marine waters were examined under various multiplicity of infection (MOI = 5.28 - 0.0017) using *Escherichia coli* AB1157 as the recipient. The *E. coli* population was reduced by as much as one to two orders of magnitude. Transduction frequencies between 10^{-6} and 10^{-2}/VLP were observed for all VLPs studied. The VLPs examined here may differ from current concepts of viruses with narrow host ranges. Such VLPs are likely to control microbial populations and promote microbial diversity by non-specific gene transfer with hosts composing a wider phylogenetic range in natural microbial populations.

Introduction

Recent studies have shown that viruses [1, 2, 4, 5-8, 10-12, 15, 18, 19, 24] and protista are the two major agents controlling microbial biomass. However, our current knowledge on viruses is limited to those that are lytic [5] and little is known about lysogens [22] in natural aquatic environments. Several workers [10, 12, 18, 19, 24] have indicated that 40-75% or more of marine bacteria contain inducible prophages. Virus-Like Particles (VLPs) in natural environments have not yet been well characterised [6, 7, 8]. Their origin, biochemical nature, infectious activity, host specificity, capability for gene transfer and fate are far from clear. At present, there is no reason to believe that VLPs in natural aquatic environments share common characteristics with viruses that are associated with bacteria such as *Escherichia coli* or enteric bacterial groups.

The present work began when some marine bacterial isolates were found to spontaneously release VLPs into the culture media [6, 7, 8]. These VLPs contain DNA and have morphological similarities to viruses [3, 5]. However, their mode of formation, mechanism of bactericidal effect and gene transfer seems to be different from any types of lysogenic or virulent cycle of bacteriophage [6, 7, 8]. The present communication will describe the basic nature of these particles and discuss implications of their presence in natural environments.

Release of VLPs from Marine Bacteria

Aerobic gram-negative marine eubacterial strains of *Agrobacterium kieliense*, *Flavobacterium* sp. I1604, Alc 096, Alc 233 and Alc 252 were used [5, 6, 7]. All strains

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possess ubiquinone-10 (CoQ10) as the sole component of coenzyme Q. 16S rRNA sequence [9] analyses indicated that, except for Alc 096, these strains belong to the Proteobacteria α subdivision. The exact phylogenic position of Alc 096 is not yet clear, since it has no known close relatives [personal communication, Akagawa-Matushita]. Cells were cultured in three litres of PPES II medium [6, 7, 8] at 25 °C in the dark with shaking (120 rpm). Culture supernatants were treated with DNase I, RNase A and phenylmethylfulfonyl fuloride (PMSF), and concentrated using a Minitan S (Millipore, USA) system with a 10kDa cutoff-membrane [8]. After filtration and ultracentrifugation, the VLPs were finally purified by CsCl-density gradient ultracentrifugation [8].

All five marine strains spontaneously released VLPs into the culture medium after prolonged incubation. Table 1 shows VLP and cell numbers after 100 hr of culture. VLPs seemed to attain equilibrium with the cells after entering a stationary phase [8]. This phenomenon is not restricted to these particular test strains. Preliminary experiments with other marine isolates, such as *Alcaligenes* (one strain), *Flavobacterium* (two strains), *Oceanospillirum* (one strain), *Vibrio* (three strains) and six unidentified strains showed similar results.

Table 1. VLP and cell number at 100 hr culture with specific reference to VLP:host bacteria ratio. Number of cells and VLPs were determined according to Børsheim [4] as described in Chiura [8]. VLP-induction frequency is given as the fraction of VLP-bearing cells in the total cell population.

Strain	Cells/ml	VLP/ml	VLP/cell No	VLP-induction frequency
A. kieliense	1.29×10^{10}	7.07×10^{8}	0.055	9.0
Alc 096	6.69×10^{8}	7.11×10^{8}	1.062	5.5
Alc 233	2.93×10^{8}	4.51×10^{8}	1.541	7.0
Alc 252	4.67×10^{9}	5.88×10^{9}	1.260	3.8
F. sp. I1604	2.32×10^{9}	1.60×10^{9}	0.689	6.4
West Mediterranean Sea*	1.94×10^{5}	5.89×10^{5}	3.050	3.1

*VLPs were obtained from oligotrophic coastal seawater (42°36' N, 8°56' E; 5 m depth; 19 - 21 °C) of the Mediterranean Sea near the marine station STARESO at Calvi, Corsica, France in June, 1996.

Table 1 also shows the VLP:cell number ratios and the spontaneous VLP induction frequencies. It is noteworthy that in the case of coliphage λ, and phage Mu, the frequency is about 0.005 [3] and 0.0001 [13] respectively. This indicates that these marine bacteria release VLPs at considerably higher frequencies. Spontaneous VLP induction frequency was found to be consistent in a seawater sample collected from oligotrophic coastal area of the west Mediterranean Sea. The frequency coincided with that found in the marine water columns [10, 18, 19, 24].

The morphological characteristics of VLPs released from the test strains are summarised in Table 2. *A. kieliense* released vesicles in which one to several spherical particles could be encapsulated. VLPs from Alc 252 had a short tail with an ellipsoid head. VLPs of Alc 233 were observed to be icosahedral. *Flavobacterium* sp. I1604 and Alc 096 were ellipsoid and spherical, respectively. Fig. 1 shows electronmicrographs of these VLPs together with typical VLPs found in the west Mediterranean Sea. Biochemical analyses indicated these particles do contain DNA resistant to DNase treatments [6, 7, 8].

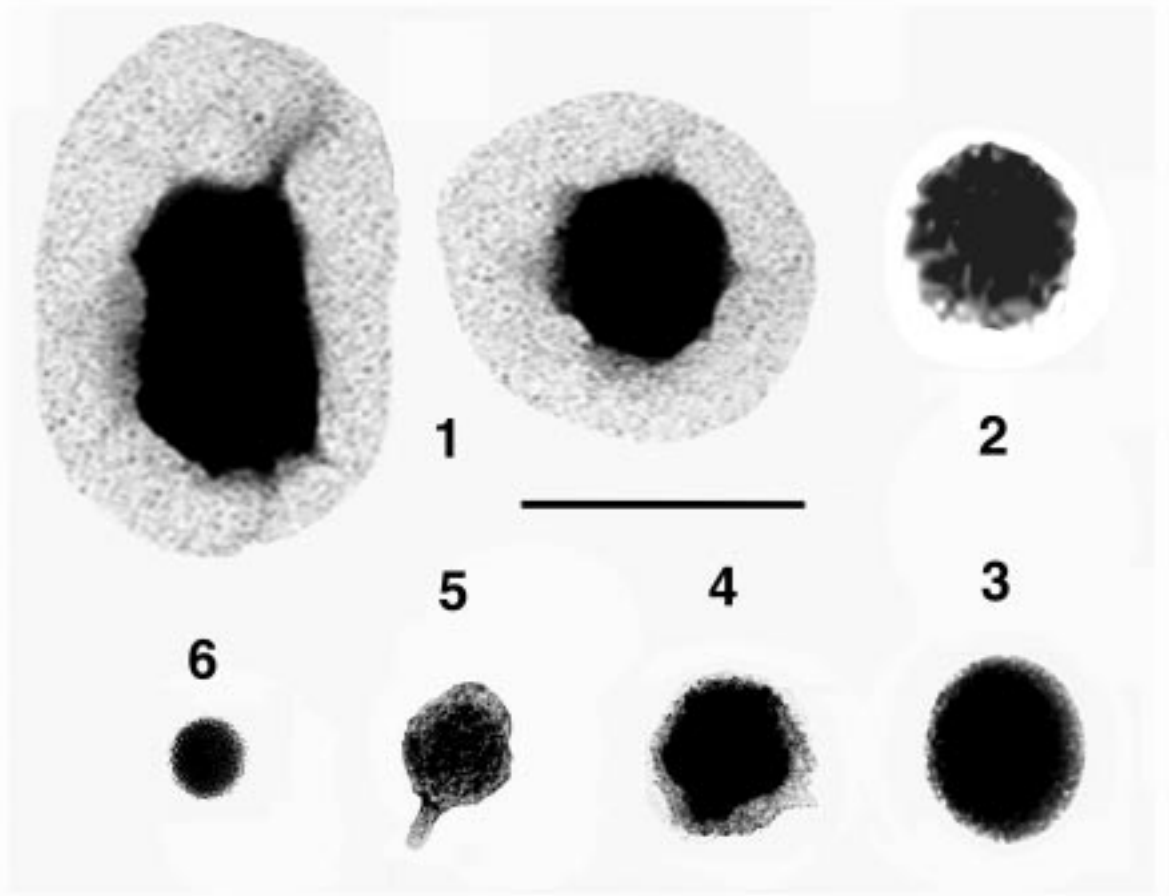

Fig. 1. Electronmicrographic images of VLPs. VLPs in culture and samples from the natural water column were examined with electron microscopy according to Børsheim et al. [3]. Following staining for 30 sec with 2 % uranyl acetate, grids were examined at x 75,000 at an accelerating voltage of 80 kV with a JEM-1200EX electron microscope (JEOL Inc., Japan) [8]. Numbers in the figure correspond to the source from which the VLP was isolated. Details are given in Table 2. (1) *Agrobacterium kieliense*; (2) West Mediterranean Sea; (3) *Flavobacterium* sp I1604; (4) Alc 233; (5) Alc 252; (6) Alc 096. Scale bar is 200 nm.

Table 2. Morphological characteristics of VLPs released from the test strains.

Source strains of VLP	**Head size in nm ± SD (n)**	**Envelope / Tail**	**# in Fig. 1**	**References**
A. kieliense	123.0 ± 3.9 (46)	+ / -	1	[6]
Alc 096	55.0 ± 2.2 (18)	- / -	6	[8]
Alc 233	78.6 ± 8.8 x 67.3 ± 6.3 (23)	- / -	4	[8]
Alc 252	78.2 ± 15.7 x 46.2 ± 10.2 (26)	- / +	5	[8]
Flavobacterium sp. I1604	85.0 ± 5.7 x 70.0 ± 2.4 (54)	- / -	3	[7]
West Mediterranean Sea	128.8 ± 58.4 x 124.9 ± 58.1 (63)	- / -	2	Unpublished [Chiura,1998]

The characteristics mentioned above indicate that some marine bacteria release VLPs as a general phenomenon after prolonged incubation [6, 7, 8]. Lytic growth of lysogenized bacteria is usually induced by UV radiation or nutrient depletion [1]. Spontaneous

induction of typical lysogens occurs at much lower frequencies [3, 13]. Therefore, this phenomenon cannot be explained by the general concept of lysogeny.

Bactericidal Effects

If these VLPs can behave as elements of gene transfer in nature, they can be expected to have certain bactericidal effects. Efficiency of plating (EOP) after exposure to various multiplicity of infection (MOI) was observed to determine the potential lethal effects of VLPs on the recipient cell, *Escherichia coli* AB1157 (see Fig. 2). VLPs concentrated and purified from the oligotrophic coastal seawater sample of the west Mediterranean Sea were

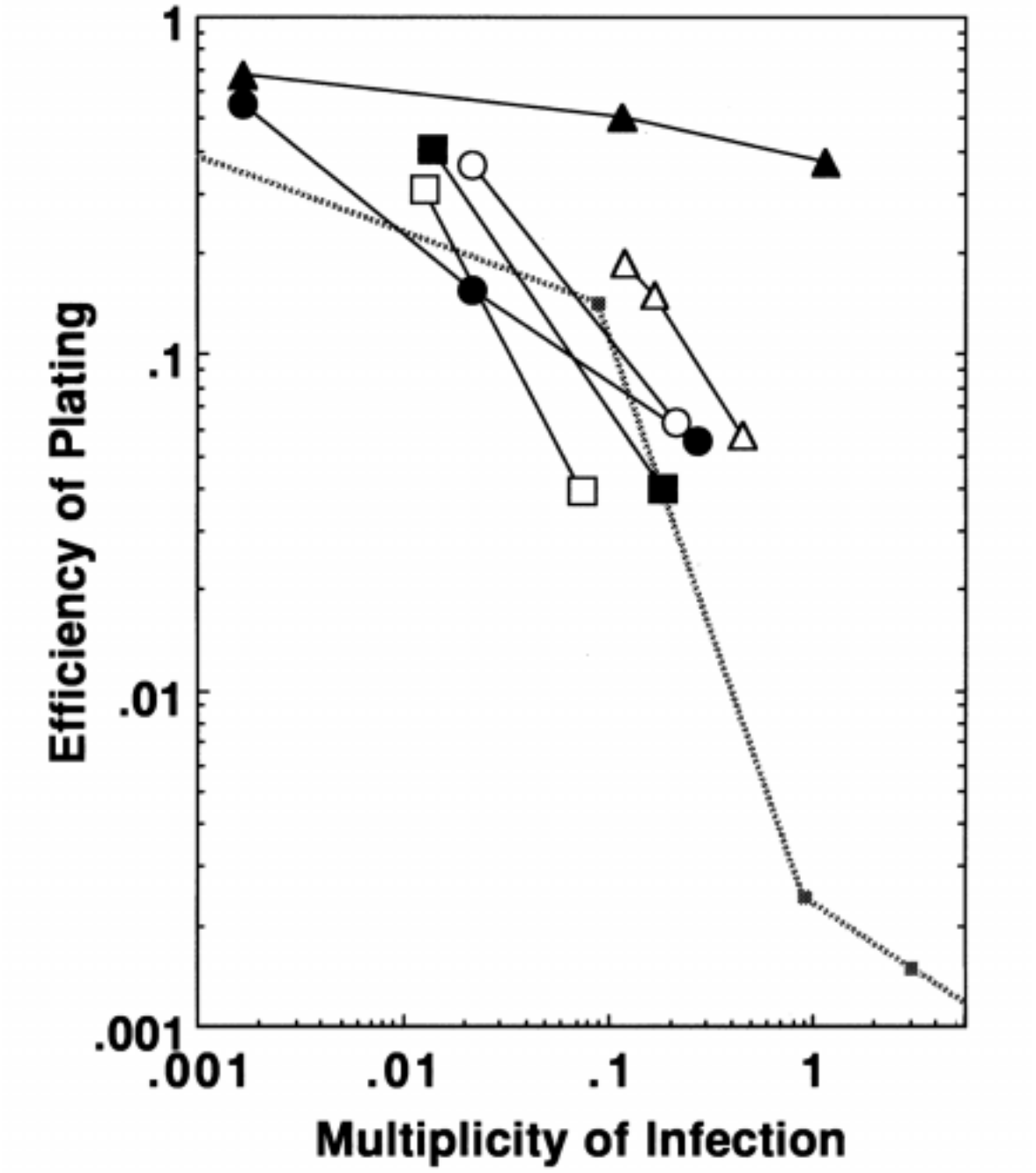

Fig. 2. Lethal effect (EOP) of VLPs on *E. coli* at various MOI (■ coliphage T4; ● *A. kieliense*; ❍ Alc 096; ■ Alc 233; □ Alc 252; ▲ *Flavobacterium* sp I1604; Δ West Mediterranean Sea).

added to VLPs from marine isolates. Purified VLPs and *E. coli* AB1157 were incubated together for 15 min at 30 °C and then examined as described in Chiura [8]. As a standard, coliphage T4 was examined in the same manner.

None of the VLPs from cultured marine isolates produced plaques on lawns of either original host bacteria or *E. coli* AB1157. VLPs from natural marine water also produced no plaques on *E. coli*. However, VLPs reduced EOP of *E. coli* AB1157 by ca 70% [8]. This was not affected by exposure of VLPs to UV-radiation for 15 min [8], and was much smaller than the effect of coliphage T4, which reduced the recipient *E. coli* population by about 1/1000 at and above MOI of 0.1 [8].

The actual processes leading to gradual cell death after exposure to VLPs is not yet clear. Electron microscopic observation indicated that multiple adhesion of VLPs onto *E. coli* cells appear to induce cell rupture without association of any apparent release of multiple VLPs.

Gene Transfer Capability of VLPs to *E. coli*

An auxotrophic mutant, *E. coli* AB1157 (F-; *thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44*) was used as the recipient bacterium for the examination of VLP-mediated gene transfer [6, 7, 8]. In addition, purified VLPs from the oligotrophic coastal seawater sample collected from the Mediterranean Sea were also examined. Threonine (thr) deficiency was not used because of its considerably high spontaneous reversion frequency (10^{-7}) [8]. For the other four markers, spontaneous reversion frequencies were below the level of detection. Freshly prepared recipient cells (ca 2×10^8 cfu/ml) were mixed with a VLP specimen to obtain a MOI of 0.1. The tube was left undisturbed at 30 °C for 15-min [8]. After incubation, cells were plated in triplicate on appropriate selection media [8].

VLPs from both cultured marine strains and natural seawater samples successfully repaired amino acids deficiencies of *E. coli* AB1157 (Table 3). Depending on the source of VLP and MOI conditions (data not shown), transduction frequency per VLP varied between 6.7×10^{-2} and 8.9×10^{-6}. No gene transfer was detected from UV-irradiated VLPs under the same conditions. DNase I pre-treatment did not affect the transfer frequency of any VLPs [6, 7, 8, Chiura (unpublished data)]. The transduction frequencies exhibited by such VLPs were considerably higher than those previously reported [12, 16, 20-23].

Table 3. VLP-mediated gene transfer of chromosomal genes (markers and map location for respective marker on recipient *E. coli* chromosome). Gene transfer frequencies are expressed per 10^7 VLPs. Values represent mean of triplicate independent experiments (three subsamples per experiment).

	Gene transferred cells per 10^7 VLPs			
Source/ Mark	**Leu, 2'**	**Pro, 6'**	**His, 44'**	**Arg, 90'**
A. kieliense	26364	21591	23500	10500
Alc 252	1065	1438	3546	1164
Flavobacterium sp. I1604	1015	1526	1336	1518
Alc 233	578	996	602	760
Alc 096	481	767	358	822
West Mediterranean Sea	450	567	384	300

It is strongly suggested that all the VLPs examined carry out generalised gene transfer since the loci of four genetic markers were distributed on the *E. coli* chromosome [6, 7, 8, Chiura (unpublished data)]. In other words, marine bacteria may release particles that contain various parts of genetic information of the host bacterium.

Conclusion

These investigations demonstrate that some marine isolates produce VLPs without artificial induction. These VLPs show bactericidal effects on *Escherichia coli*, which belongs to a phylogenically different group. Furthermore, these particles are capable of intergeneric generalised gene transfer. It is also suggested that at least some of the VLPs in natural seawater may share similar characteristics. These results are not explained by the general feature of lytic or lysogenic cycles of viruses infectious to bacterial cells. The nature of this type of VLP, the mechanisms of bactericidal effects and gene transfer, and the actual role played in natural environments need to be clarified.

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