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# Generalized gene transfer by virus-like particles from marine bacteria

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ABSTRACT: Spontaneous VLP (virus-like particle) production and VLP-mediated gene transfer into *Escherichia coli* AB1157 as recipient was demonstrated. Five marine isolates (Alc 096, Alc 233, Alc 252, *Agrobacterium kieliense* and *Flavobacterium* sp. I1604) were investigated for their potential to produce VLP as well as for the gene transfer capability of these VLPs to the *E. coli* recipient. These strains are classified as ubiquinone-10-possessing marine bacteria (Q10MB) in the 16s-rRNA Superfamily IV VLPs were obtained from 100 h cultured broth of all strains examined. VLP-host ratio after 100 h growth culture was: Alc 233, 1.54; Alc 252, 1.26; Alc 096, 1.06; *Flavobacterium* sp. I1604, 0.69; and *A. kieliense*, 0.06. These ratios were smaller than those found in the marine environment. However, the spontaneously produced VLP number can be considered as high because the reported numbers are relatively low from coliphage λ (0.005) and phage Mu (~0.0001). VLP-mediated gene transfer was examined using an auxotrophic mutant of *E. coli* (AB1157) with 4 amino acid deficiencies (*leu, pro, his, arg*) as recipient at multiplicity of infection (MOI) of 0.1. Through this treatment, VLPs showed lethal effect on the recipient. The survival rate of control was: Alc 096, 7%; Alc 252, 8%; *A. kieliense*, 17%; *Flavobacterium* sp. I1604, 31%; and Alc 233, 40%. At the same time, all the purified VLPs derived from these 5 strains successfully transferred genes to rescue genetic defects of the recipient. Overall average efficiency of VLP-mediated gene transfer at MOI of 0.1 was estimated to be between $2.62 \times 10^{-3}$ and $3.58 \times 10^{-5}$ per VLP particle. Loci of employed genetic markers were dispersed on the *E. coli* chromosome with mutual distance of 121, 1154, 1397 and 364 kb between them. Since VLPs from different sources showed similar gene transfer efficiency in respect to the genetic marker rescued, it is suggested that VLPs from Q10MB transferred genes as generalized transduction. These results indicate that the VLPs produced by certain marine bacteria may be an important element for both non-specific generalized horizontal gene transfer towards a broad range of bacterial hosts and population control in the marine environment.

KEY WORDS: Virus-like particles (VLPs) · Generalized transducing ability · Horizontal gene transfer · Transductants · Bactericidal effect · Marine bacteria · Ubiquinone-10

## INTRODUCTION

Viruses (or virus-like particles, VLPs) in aquatic environments are acknowledged to be general constituents of the ecosystem (Bergh et al. 1989, Børsheim et al. 1990, Ogunseitan et al. 1990, Hara et al. 1991, Wommack et al. 1992, Børsheim 1993, Fuhrman & Suttle 1993, Paul et al. 1993, Mathias et al. 1995). Major postulated roles for phages (VLPs) in the aquatic environment are bacterial population control and gene transfer *in situ* (Miller & Sayler 1992). Recently, the possible functions of viruses or VLPs in controlling bacterial mortality and the reduction of microbial production have been reported (Proctor & Fuhrman 1990, Suttle et al. 1990, Maruyama et al. 1993, Nagasaki et al. 1993, Suttle & Chan 1994, Weinbauer & Peduzzi 1994, Hennes & Simon 1995, Mathias et al. 1995). Known modes of gene transfer are plasmid-mediated conjugation, genetic transformation, and phage-mediated transduction (Gauthier & Breittmayer 1990, Ambile-Cuevas

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& Chicurel 1992). Horizontal gene transfer is assumed to be important for the evolution and genetic diversity of natural microbial communities and is therefore an important phenomenon to be understood (Saye & Miller 1989). However, it is well known that bacterial cells generally restrict their acceptance of foreign genetic materials (Streips & Yasbin 1991). Although 'trans-kingdom' gene transfer has been reported (Stachel et al. 1986, Heineman & Sprague 1989, Zambryski et al. 1989, Sikorski et al. 1990), the occurrence of gene transfer between different bacterial species and genera in nature is so far known to be mediated by plasmids (Don & Pemberton 1981, Brisson-Noel et al. 1988, Schafer et al. 1990, Mazodier & Davies 1991). Promiscuous gene transfer, when observed, is predominantly mediated by plasmids (Lorenz & Wackernagel 1994). Hence, virus-mediated gene transfer (transduction) has been considered to be a factor of minor importance for genetic diversity and evolution in the natural microbial community. Only recently has transduction become apparent as a potentially important means for the redistribution of genetic information in natural microbial habitats (Miller & Sayler 1992, Ripp et al. 1994, Schicklmaier & Schmieger 1995).

During a study on properties of marine bacterial endonucleases (Chiura et al. 1988, 1992a, b), excretion of VLPs bearing nucleic acid into the broth was incidentally observed from the strains used for the investigation (Chiura & Takagi 1994, Chiura et al. 1995). These rod-shaped marine strains were classified as members of the Superfamily IV by 16s-rRNA analysis (De Ley 1991), and by having ubiquinone-10 as the sole component of coenzyme Q (abbreviated as ubiquinone-10-possessing marine bacteria: Q10MB). Spontaneous releases of VLPs from eubacterial strains have been seldom reported, and are considered as being quite unusual, although some similar phenomena in archae- and photosynthetic bacteria were mentioned by Reiter et al. (1987), Shaefer et al. (1974), Solioz & Marrs (1977), Wall et al. (1975), Wood et al. (1989), and Schleper et al. (1992). Recently, a high occurrence of spontaneously induced temperate phages together with high frequency of a generalized transducing trait in natural isolates of *Salmonella* have been reported (Schicklmaier & Schmieger 1995). The authors pointed out that the potential for phage-mediated gene transfer may be much higher than expected.

VLPs from *Flavobacterium* sp. I1604 belonging to Q10MB have been shown to mediate chromosomal gene transfer in *Escherichia coli* with lethal effects (Chiura et al. 1995). Hence, VLPs derived from several marine bacteria which were not related to recipient *E. coli*, at least at the family level, were investigated. The purpose of this study was to determine if VLPs derived from some Q10MB are on the one hand capable of controlling bacterial population diversity and on the other capable of mediating gene transfer.

## MATERIALS AND METHODS

**Bacterial strains as the source of VLPs and gene transfer recipient bacteria.** Aerobic Gram-negative marine eubacterial strains of *Agrobacterium kieliense*, Alc 096, Alc 233, Alc 252 and *Flavobacterium* sp. I1604 were used in this study as sources of VLPs. Sampling and isolation sites are summarized in Table 1. *A. kieliense* IAM 12618 was obtained from the IAM Culture Collection, Center for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, the University of Tokyo, Bunkyo-ku, Tokyo, Japan. This strain is classified in the rRNA Superfamily IV (the α–subdivision) in the class Proteobacteria, however there are no known close relatives (De Ley 1991). Rüger & Höfle (1992) also indicated that the strain could not be placed in a subdivision of the genus *Agrobacterium*. Strains Alc 096, Alc 233, and Alc 252 were obtained from M. Akagawa-Matsushita, Minaikyushu University. Based on rRNA-DNA hybridization experiments (Akagawa-Matsushita pers. comm.), Alc 096 had been shown to have no relationship to any members of rRNA Superfamilies I, II, III and IV, and therefore its phylogenetic position is not yet determined. The strains Alc 233 and Alc 252 have been shown to belong to the rRNA Superfamily IV, however they are not closely related to each other and there are

Table 1 Ubiquinone-10-possessing marine bacterial (Q10MB) strains, sampling site, material and source (year). They are Gram-negative, rod-shaped, aerobic, marine eubacteria. 'Alc' numbers are arbitrary, given by Akagawa-Matsushita. Although their phylogenic position was determined using 16s-rRNA analysis (De Ley 1991), they do not have strain nomenclature

| Strain | Sampling site | Material | Source |
|---|---|---|---|
| *Agrobacterium kieliense* | Kiel Bay, Baltic Sea | Seawater | M. Akagawa-Matsushita (1988) |
| Alc 096 | Iou-Tou Is., Pacific Ocean | Seawater | M. Akagawa-Matsushita (1988) |
| Alc 233 | Chiba, Japan | Seaweed | M. Akagawa-Matsushita (1988) |
| Alc 252 | Kanagawa, Japan | Seaweed | M. Akagawa-Matsushita (1988) |
| *Flavobacterium* sp. I1604 | Indian Ocean | Seawater | U. Simidu (1983) |

no known close relatives (Akagawa-Matsushita pers. comm.). As *Escherichia coli* belongs to rRNA Superfamily I (γ-subdivision) in the class Proteobacteria (De Ley 1991), these 5 strains are not related to *E. coli*, at least at the family level. *Flavobacterium* sp. I1604 was donated from the natural isolate culture collection of the Ocean Research Institute, the University of Tokyo, Nakano-ku, Tokyo, Japan. The strain *Flavobacterium* sp. I1604 has ubiquinone-10 (Q10) and its mole percent guanine plus cytosine content (mol% G+C) in DNA is 65.2% (Akagawa-Matsushita pers. comm.). This strain is probably not a member of the *Flavobacterium* because of its lack of menaquinones. Although its phylogenetic position is still unknown, the presence of Q10 suggests that it should be assigned to the rRNA Superfamily IV in the class Proteobacteria (abbreviated as ubiquinone-10-possessing marine bacteria, Q10MB). All the species in the rRNA Superfamily IV have Q10 as coenzyme Q (CoQ) with few exceptions (Collins & Jones 1981, Yokota et al. 1992).

*Escherichia coli* AB1157 was obtained from the National Institute of Genetics (Shizuoka, Japan), and has the following genetic features: F-; *thr*-1 *leuB*6 *thi*-1 *lacY*1 *galK*2 *ara*-14 *xyl*-5 *mtl*-1 *proA*2 *his*-4 *argE*3 *rplsL*31 *tsx*-33 *supE*44. *E. coli* AB1157 was used as the recipient bacterium for VLP-mediated gene transfer.

**Culture conditions.** PPES II [modified seawater broth: 0.1% proteose peptone no. 3, 0.2% polypeptone, 0.1% yeast extract and 0.1% Bacto soyton in Jamarin S (artificial seawater, Jamarin Laboratory), pH 7.5] (Chiura et al. 1988) was used as nutrient medium for the culture of marine strains at 25°C and the liquid culture (3 l) was shaken at 120 rpm. LB [Luria Bertani medium: 1% polypeptone, 0.5% yeast extract, 1% sodium chloride in distilled and deionized water (DDW), pH 7.5] (Sambrook et al. 1989) was used to culture *Escherichia coli* at 30°C and liquid cultures were shaken at 120 rpm. Bacteria were grown in the dark during experiments to eliminate any light effects.

**Selection medium.** For selection of VLP-mediated gene transferred *Escherichia coli*, minimal media (MM) after Davis (in Difco Manual 1985) supplemented with 3 out of 4 amino acids, namely, leucine, proline, histidine, and arginine were used. MM contained 10% Davis salt solution [0.2% $KH_2PO_4$, 0.7% $K_2HPO_4$, 0.1% $(NH_4)_2SO_4$, 0.05% sodium citrate], 0.01% $MgSO_4$, 0.2% glucose and 1 mg $l^{-1}$ thiamine. Amino acids of 20 mg $l^{-1}$ each were supplemented to the selection media.

**Preparation of VLPs.** VLPs were isolated and purified as previously described from prolonged culture broth of respective strains (Chiura et al. 1995). In brief, culture filtrate was obtained by centrifugation (7500 × *g*, 40 min) from 100 h cultured marine strains in PPES II broth at 25°C with shaking. Cell washing was done with 0.5 M NaCl and 1 mM EDTA (Chiura et al. 1995) by centrifugation (7500 × *g*, 40 min). DNase I, RNase A and phenylmethylsulfonyl fluoride (PMSF) (Sigma, USA) were added to the culture filtrate as 1 μg $ml^{-1}$, 1 μg $ml^{-1}$ and 100 nM and kept at 25°C overnight. The culture filtrate was then passed through a 0.2 μm membrane filter (Millipore, USA) and concentrated to ca 50 ml using a Minitan S (Millipore, USA) system with 10 kDa cut-off filter.

The concentrated culture filtrate was filtered again through 0.45 μm and 0.22 μm membrane filters, then centrifuged at 80000 × *g* for 30 min using a Beckman Preparative Ultracentrifuge L8M with 55.2Ti rotor to pellet VLPs. The obtained pellet was resuspended in 100 μl of TBT buffer (100 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$) and purified by CsCl-density gradient ultracentrifugation (Davis et al. 1980).

**Enumeration of cells and VLPs.** Prior to the harvest of the culture filtrate, a viable count of cells and number of VLPs was investigated. A viable count of cells was determined on PPES II solid medium at 25°C. The number of VLPs was determined according to Børsheim et al. (1990) for culture filtrates with 0.22 μm membrane (Millex-GS, Millipore, USA) filtration, because the marine strains used as VLP sources secreted a murein-like substance during culture. Since this murein-like substance could not be removed even with brief centrifugation and dilution, direct counts of VLPs from culture broth on the electronmicroscopic grid were impractical. Therefore enumeration was conducted for the culture filtrate. Following staining for 30 s with 2% uranyl acetate, grids were examined at ×75000 at an accelerating voltage of 60 kV with a JEM-1200EX electron microscope (JEOL Inc., Japan). Eye fields were randomly selected and counted until the total count exceeded 250 VLPs. The size of the eye field was used to keep track of the area.

**VLP-mediated gene transfer protocol to *Escherichia coli*.** In order to ensure reproducible physiological condition of the recipient *E. coli* AB1157, the bacterium was cultured at 30°C by shaking (120 rpm) until a cell density of $4 \times 10^8$ cfu $ml^{-1}$ was attained, then glycerin (Merck, Germany) was added to the culture to a final concentration of 7%, and the culture dispensed as 2 ml aliquots. Seed culture aliquots were frozen immediately in liquid nitrogen and kept at −85°C until use. For gene transfer experiments, a 2 ml aliquot of frozen seed culture was mixed with 3 ml of fresh LB broth in an L-shaped test tube and grown to mid-exponential phase at 30°C with shaking, then centrifuged at 5000 × *g* in a Kubota RT20000 refrigerated centrifuge using an RA3 rotor (Kubota, Japan) and suspended in 5 ml of TBT buffer. The cell suspension thus obtained gave a viable count of ca $2 \times 10^8$ colony forming units (cfu) $ml^{-1}$. One ml of this suspen-

sion was mixed with a VLP specimen to obtain a multiplicity of infection (MOI) of 0.1. The tube was left undisturbed at 30°C for 15 min. After incubation, cells were washed with Davis salt solution and finally suspended in 1 ml of the same solution. This mixture was plated in triplicate on appropriate selection media and incubated for 2 d at 30°C. Five controls were included: (1) recipient cells with TBT buffer instead of VLPs to determine spontaneous revertant rate; (2) ultraviolet light (UV)-inactivated VLPs and recipient cells; (3, 4) VLPs with/without UV inactivation with no added recipient cells; and (5) contained VLPs, recipient cells and DNase I (10 µg $ml^{-1}$) to exclude the possibility of transformation. For UV inactivation, a VLP suspension was placed in a Petri dish, filled with water to 2 mm, and the suspension of VLPs was irradiated for 15 min with a 15 W UV sterilizing lamp (Hitachi, Japan) from 30 cm. As a reference for UV inactivation, coliphage T4 was treated under the same conditions, and as a result the plaque forming ability of T4 was reduced by 7 orders of magnitude.

## RESULTS AND DISCUSSION

### Bacterial growth and VLP production

All 5 marine strains (Q10MB) spontaneously released VLPs into the culture medium after prolonged incubation. None of the VLPs obtained in the present study gave plaques on lawns of both the original host bacteria and recipient *Escherichia coli*. VLP and cell numbers and ratios after 100 h culture are summarized in Table 2. In descending order, VLP:host bacteria ratios were: Alc 233, 1.54; Alc 252, 1.26; Alc 096, 1.06; *Flavobacterium* sp. I1604, 0.69; and *Agrobacterium kieliense*, 0.06. Although direct comparison of these ratios with values from the natural water column is not appropriate, it should be pointed out that the highest ratio was far smaller than values found in the natural aquatic environment (Bergh et al. 1989, Børsheim et al. 1990, Proctor & Fuhrman 1990, Hara et al. 1991, Hennes & Suttle 1995, Mathias et al. 1995). Incidentally, spontaneously induced coliphage λ allowed one to estimate a virus-host ratio of 0.005 (Birge 1994). Phage Mu lysogens release phages spontaneously at a low level virus-host ratio of ~0.0001 but are not induced by treatment with UV or other DNA-damaging agents (Howe & Bade 1975, Ljungquest & Bukhari 1977, Howe 1987). Although the values observed in this study were biased due to filtration, marine strains have shown considerably high rates of spontaneous VLP induction.

Lytic growth of lysogenized bacteria in nature would be induced by a factor such as UV light or nutrient depletion (Ackermann & DuBow 1987). Though the influence of UV-visual light irradiation on the lysogens' induction has been reported to be small (Wilcox & Fuhrman 1994), bacteria were grown in the dark during experiments to eliminate any light effects. Almost all nutrient in the broth (dissolved total carbohydrate and protein content) was consumed by the bacteria at about 18 h after starting the culture (data not shown), so it is likely that initiation of a starvation state of the host triggers VLP production. In certain aquatic environments, as many as 45% of isolates were lysogens (Ogunseitan et al. 1990, Miller et al. 1992). In addition, more than 90% of natural isolates of *Salmonella* have been reported to be lysogenized (Schicklmaier & Schmieger 1995). Marine strains used in this study are most probably lysogens.

The spontaneous release of VLPs has been reported from archaebacteria, *Methanococcus voltae* (Wood et al. 1989), *Sulfolobus* (Zilling et al. 1988), and the photosynthetic bacterium *Rhodopseudomonas capsulata* (Wall et al. 1975, Solioz & Marrs 1977), but the phenomenon has not been considered ubiquitous in nature. However, preliminary experiments to examine spontaneous VLP production using marine isolates such as *Alcaligenes* (1 strain), *Flavobacterium* (2 strains), *Oceanospillirum* (1 strain), *Vibrio* (3 strains) and 6 unidentified strains showed positive results (Chiura unpubl. data). All of these strains were isolated from an ordinary, not extreme, marine environment (Table 1). Recently, high occurrence of spontaneously induced temperate phages were found in natural isolates of *Salmonella* with a high generalized transducing trait, which suggested a high rate of existence of spontaneously induced phage production and higher potential of phage-mediated gene transfer in a natural habitat (Schicklmaier & Schmieger 1995). Therefore, spontaneous production of VLP might be a common feature shared among certain marine bacteria, at least in the strains of Q10MB. Such VLP-host coexistence allows us to speculate that naturally occurring gene exchange processes may occur in the marine microbial community.

Table 2. VLP and cell number at 100 h culture with specific reference to VLP:host bacteria ratio

| Strain | Cells $ml^{-1}$ | VLP $ml^{-1}$ | VLP:cell no. |
|---|---|---|---|
| *Agrobacterium kieliense* | $1.29 \times 10^{10}$ | $7.07 \times 10^{8}$ | 0.055 |
| Alc 096 | $6.69 \times 10^{8}$ | $7.11 \times 10^{8}$ | 1.062 |
| Alc 233 | $2.93 \times 10^{8}$ | $4.51 \times 10^{8}$ | 1.541 |
| Alc 252 | $4.67 \times 10^{9}$ | $5.88 \times 10^{9}$ | 1.260 |
| *Flavobacterium* sp. I1604 | $2.32 \times 10^{9}$ | $1.60 \times 10^{9}$ | 0.689 |

### Morphology of VLPs

Characteristic features of obtained VLPs are summarized in Table 3. VLPs from *Agrobacterium kieliense* were especially characterized for an envelope structure of varying length (0.8 to 1.5 µm) in which 1 to several spherical particles (number of particles inside ± SD, 5.1 ± 2.8 particles; examined number of envelopes, n = 60) were encapsulated (Chiura & Takagi 1994). Though some eukaryotic viruses, such as the influenza virus, have envelopes, this is not a common feature for prokaryotic viruses. RNA phage ø6 of *Pseudomonas* is a typical example of an encapsulated prokaryotic virus whose particle is incorporated into the recipient cell during infection (Calender 1988). The gene transfer agent (GTA) of *Rhodopseudomonas* is reported to be encapsulated (Wall et al. 1975, Solioz & Marrs 1977). Transformasomes from *Haemophilus* are reported to produce membranous material which is capable of encapsulating exogenous DNA in the surroundings of the cell (Kahn et al. 1983). However nothing is known about the relationship between those reported membranous encapsulated particles and the particles studied in this study.

VLPs from Alc 252 had a short tail (ca 25 nm) structure with an ellipsoid head. VLPs of Alc 233 were observed to be icosahedrons. *Flavobacterium* sp. I1604 gave ellipsoid VLPs (Chiura et al. 1995) and Alc 096 were almost complete spherical particles. In terms of head size, VLPs from *Agrobacterium kieliense* were classified in the largest category of head size class and VLPs from the other 4 strains were in the most abundant head size class found in the sea, namely 60 to 80 nm (Børsheim 1993).

### VLP-mediated gene transfer experiment to *Escherichia coli* AB1157

2,4-Dichlorophenoxyacetic acid utilization (2,4-D) and some chromosomal gene transfer mediated by VLPs derived from *Agrobacterium kieliense* and *Flavobacterium* sp. I1604 to recipient *Escherichia coli* AB1157 have been demonstrated earlier (Chiura & Takagi 1994, Chiura et al. 1995). In order to examine the possibility of non-specific VLP-mediated gene transfer, sources of VLPs were extended to other Q10MB, namely Alc 096, Alc 233 and Alc 252. *E. coli* AB1157 is an auxotrophic mutant with 5 amino acid deficiencies (*thr, leu, pro, his, arg*), however *thr* was not used because of its considerably high spontaneous reversion frequency ($\sim 10^{-7}$). For the other 4 markers, spontaneous reversion frequencies were found to be below the level of detection. Experimental control of VLP specimens (UV +/–) plated without adding recipient cells gave no colony formation by plating. DNase I did not affect the gene transfer frequency of any VLP sources. Observed gene transfer frequency with DNase was between 89 and 112% when compared with the efficiency without DNase, defined as 100%.

Experiments were designed to obtain the expected MOI of 0.1. Observed MOI for each type of VLP were: *Agrobacterium kieliense*, 0.22; Alc 096, 0.18; Alc 233, 0.14; Alc 252, 0.13; and *Flavobacterium* sp. I1604, 0.12. Efficiency of plating (EOP) after exposure to MOI of 0.1 was used to observe the lethal effect of VLPs to recipient cells. These VLPs showed lethal effects on recipient *Escherichia coli* by reducing EOP up to 1 order of magnitude. Observed EOP (±SD) for each VLP was as follows: Alc 096, $6.90 \pm 3.1 \times 10^{-2}$; Alc 252, $7.80 \pm 5.2 \times 10^{-2}$; *A. kieliense*, $1.73 \pm 1.6 \times 10^{-1}$; *Flavobacterium* sp. I1604, $3.06 \pm 0.33 \times 10^{-1}$; and Alc 233, $4.03 \pm 0.45 \times 10^{-1}$. Under the same MOI and experimental conditions (MOI = 0.09, 30°C, 15 min adsorption), the lethal effect of coliphage T4 on *E. coli* was $2.44 \times 10^{-5}$ (data not shown). Such low virulence for these VLPs explains why we could not obtain any plaques on the original host strains and *E. coli*. The extent of VLP lethality was comparable to that of piocin R, a bacteriocin produced by *Pseudomonas* (Amako & Yasunaka 1979). Although these VLPs had a lower killing rate on *E. coli* than the typical lytic coliphage T4, they showed lethality on bacteria of a different phylogenic group. Hence, they can be taken into account as contributing to the 'phage-induced mortality' in the natural water column (Weinbauer & Peduzzi 1994, Hennes & Simon 1995).

Amino acid deficiencies of *Escherichia coli* were successfully repaired through the gene transfer experiment using VLPs as summarized in Table 4. When UV-irradiated VLPs were used as gene transfer mediators,

Table 3. Source strains, average head size in nm (±SD, n in parentheses), and presence/absence of envelope and tail of VLPs

| Source strains of VLP | Head size ± SD, nm (n) | Envelope / Tail | Source |
|---|---|---|---|
| *Agrobacterium kieliense* | 123.0 ± 3.9 (46) | +/– | Chiura & Takagi (1994) |
| Alc 096 | 55.0 ± 2.2 (18) | –/– | This study |
| Alc 233 | 78.6 ± 8.8 × 67.3 ± 6.3 (23) | –/– | This study |
| Alc 252 | 78.2 ± 15.7 × 46.2 ± 10.2 (26) | –/+ | This study |
| *Flavobacterium* sp. I 1604 | 85.0 ± 5.7 × 70.0 ± 2.4 (54) | –/– | Chiura et al. (1995) |

Table 4. VLP-mediated gene transfer of chromosomal genes. Markers and map location for respective marker on recipient *Escherichia coli* chromosome. Gene transfer frequencies are expressed per $10^7$ VLPs. Values represent mean of triplicate independent experiments (3 subsamples per experiment). No gene transfer frequencies were detected from selection plates to which UV irradiated VLP were used as the VLP sources. DNase I did not affect the gene transfer frequency of any VLP sources

| Mark | Gene transferred cells per $10^7$ VLPs | | | | |
|---|---|---|---|---|---|
| Source: | *Agrobacterium kieliense* | Alc 252 | *Flavobacterium* sp. I1604 | Alc 233 | Alc 096 |
| Leu, 2' | 26364 | 1065 | 1015 | 578 | 481 |
| Pro, 6' | 21591 | 1438 | 1526 | 996 | 767 |
| His, 44' | 23500 | 3546 | 1336 | 602 | 358 |
| Arg, 90' | 10500 | 1164 | 1518 | 760 | 822 |

no gene transfer frequencies were detected. Although transfer efficiencies for respective markers varied upon applied VLP source, distinctive preference of marker transfer was not observed among markers. At a MOI of 0.1, *leu*, *pro*, *his* and *arg* markers on the *E. coli* chromosome exhibited gene transfer frequencies ranging between $2.62 \times 10^{-3}$ and $3.58 \times 10^{-5}$ per VLP. Transducing frequencies of these VLPs were found to be higher by 4 to 7 orders of magnitude than those reported for naturally isolated transducing phages (Saye et al. 1990, Ripp et al. 1994). The marine strains used as VLP source produced a murein-like substance in the broth which was difficult to remove from VLP specimens even after density gradient ultracentrifugation. It is speculated, however, that such a concomitantly isolated murein-like substance together with VLP might play an important role during the gene transfer process. It may stabilize VLP adhesion at a certain receptor site of the recipient cell to promote further gene transfer. Some analogous features of the substance might be membranous materials found during the transformation process in *Bacillus* (te Riele & Venema 1984), *Haemophilus* (Kahn et al. 1983) and *Neisseria* (Doward et al. 1989).

Consistent gene transfer frequencies for every genetic marker were displayed by VLPs from the same source. Since the loci of employed genetic markers did not come across closely but were dispersed on the *Escherichia coli* chromosome, it is suggested that all the VLPs examined here carry out generalized gene transfer. As described above, efficiency of lethality was different from source to source and no correlation was found between lethality and gene transfer.

Bacterial conjugation is considered to be the most widespread mechanism of gene exchange in the microbial community (Birge 1986) and this process includes cell to cell contact. Thus, unless the density of the recipient is substantial, one would not expect a high frequency of conjugative gene transfer (Levin & Lenski 1983). It has been reported that under starvation conditions found in the aquatic habitat the establishment of lysogeny is favoured (Romig & Brodetsky 1961). Viruses may adsorb to clay minerals and other particles, which appears to protect them against degradation or loss of infectivity from UV exposure over long periods of time (Stozky 1980). An important point to mention is that viruses may persist in the environment under such conditions. This increases the probability of transduction occurring even in environments where the cell density is low. In fact, bacteriophages adsorbed on clay minerals may serve as reservoirs of bacterial DNA in soil and other natural habitats (Stozky 1989). Under the coexistence of lysogenic viruses and bacteria, viruses may be seen as partners of coevolution for the supply of complementary genetic source to the bacterial community (Levin & Lenski 1983). The packaging of genetic material in a transducing particle probably represents an evolutionary survival mechanism for bacterial genes (Stozky 1989, Veal et al. 1992).

It is generally accepted that the accessible host range for a virus is restricted to the same species, and, even if possible, among the receptors of related species (Calender 1988, Kokjohn 1989, Børsheim 1993, Fuhrman & Suttle 1993, Birge 1994). The surface features of VLPs might have been one of the factors that determined the uptake of nucleic acids by recipient cells (Hirsch 1990, Dreiseikelmann 1994). Accordingly, virus-mediated gene transfer has not been considered as an important process for redistribution of genetic information. Recently, sufficient evidence has accumulated to exploit the idea that transduction is a meaningful way of gene exchange, being more important in natural ecosystems than has been traditionally envisioned (Novick et al. 1986, Kokjohn 1989, Saye & Miller 1989, Stozky 1989, Saye et al. 1990, Miller et al. 1992, Schicklmaier & Schmieger 1995). The present results imply that spontaneously produced VLPs are important factors for non-specific gene transfer in the marine environment. These VLPs must have interaction with receptors on the recipient bacteria that are not closely related in terms of phylogeny. Hence receptors for such VLPs examined here must share common features in a wide range of different bacterial genera. Phage P1 is also known for its wide host range (Birge 1986, Yarmo-

linsky & Sternberg 1988). P1 mutants with extended host range were used to facilitate gene mapping of bacterial hosts, so it is still possible to suspect the existence of viruses (VLPs) with a wide host range in nature (Calender 1988).

This study demonstrated that some marine isolates produce VLPs without artificial induction. These VLPs showed bactericidal effect on *Escherichia coli*, which belongs to a totally different genus compared to the marine bacterial sources of VLP. Furthermore, such VLPs are capable of intergeneric generalized gene transfer. The importance of this observation is that this is the first demonstration of interspecific and/or intergeneric VLP-mediated gene transfer found among marine bacteria and enteric bacteria. This study implies the existence of spontaneous VLP production mechanism and gene transfer kinetics in the marine environment. The results also suggest that there is a high potential of population control by such VLPs. The source strains of VLPs were not collected from extreme sites, such as hot vents or hypersaline environments, but ordinary marine environments (Table 1). Such facts, together with the results of the present experiment, strongly suggest the prevalent existence of an interspecific and/or intergeneric natural genetic transfer system.

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