Broad-Host Range Gene Transporter Particles Produced by *Aliivibrio fischeri*

HIROSHI XAVIER CHIURA1,2,*, NAMI UCHIYAMA1†, and KAZUHIRO KOGURE†

1Marine Microbiology Laboratory, Department of Marine Ecosystems Dynamics, Ocean Research Institute, University of Tokyo, 1–15–1 Minamidai, Nakano-ku, Tokyo 164–8639, Japan; and 2Arbeitsgruppe Allgemeine Mikrobiologie, Zentrum für Anatomie und Zellbiologie, Medizinische Universität Wien, Währingerstr. 10, Wien A-1090, Austria

(Received August 5, 2009—Accepted October 20, 2009—Published online November 12, 2009)

*Aliivibrio fischeri* NCIMB1281† (basonym, *Vibrio fischeri*) spontaneously started broad-host range vector particle (AVP) production by budding from the logarithmic phase, and stabilised at around 7.0×10−7.4×1011 particles mL−1 without any accompanying change in the host population. AfVPs had a spherical shape and varied in diameter from 18.1 to 159.2 nm [median±SD, 58.4±11.9 nm, n=528], with 95.1% between 30.2 and 84.6 nm in diameter exhibiting a normal distribution. Their buoyant density and DNA content ranged from 1.3607 to 1.3980 g cm−3, and 17.3 to 95.3 kbp, respectively. Regardless of UV treatment, AfVPs enhanced the efficiency of plating 116–136% at a multiplicity of infection of ca. 140 in *Escherichia coli* AB1157. Generalised transduction was observed with a frequency of between 10−4 and 10−6 cells per AfVP without UV treatment. Upon infection, the particle membrane remained outside the recipient cell, and a string-like structure coated with a fibrous proteinaceous-like material was present. The growth of the *E. coli* transductant (AIV-E-trans) reached a maximum of ca. 415% that of the parental *E. coli* recipient. AfIV-E-trans acquired the ability to produce budding particles.

**Key words:** broad-host range, horizontal gene transfer (HGT), serial transduction, *Aliivibrio fischeri*, VLP(s)

In environmental samples, particles similar in morphology to viruses are called virus-like particles (VLPs). Although viroplankton is the most abundant class of plankton in a variety of aquatic habitats (2, 12, 21, 23, 30, 32, 33), studies on its behaviour in the environment are scarce. VLP-mediated transduction among microbes has become of interest to researchers in microbial ecology (12, 33, 34), because of the possible impact VLPs could have on horizontal gene transfer (HGT). Virus-mediated HGT is considered one of the most important components of evolution and the genetic diversity in natural microbial communities, since virus-mediated gene transfer has had more effect on microbial populations than was previously considered (18). HGT for various gene clusters, such as drug resistance genes, has recently been reported based upon sequence analyses (16, 20). However, assessment of the degree of transduction in natural environments is still limited by insufficient experimental systems. The development of a technique of isolating VLPs from the environment may lead to new findings regarding gene transfer mediators (28).

We have demonstrated that VLPs released spontaneously into culture medium from some marine bacterial isolates show a gene transfer capability, with a lethal effect on recipients distantly related to their original host (3–5). These VLPs contain DNA, and are similar morphologically to viruses (3–5). We have proposed calling such particles “broad-host-range vector particles” (VPS) because of their extraordinary properties. VLPs only become apparent on electron microscopic (EM) observation, which reveals budding particles generated using a recipient different from the sampled habitat. Hence, the proportion of VPSs in the VLP fraction of environmental samples is unclear. VPs are postulated to randomly encapsulate a certain length of host chromosome through serial transductions (9). Regardless of UV irradiation, VPs exhibit a mildly lethal effect (maximum, 10%) towards a taxonomically broad range of recipients without plaque formation. VPs (3–10) do not fit the current concept of a “virus” with a narrow host range (1). Although the DNA-encapsulating system of VP is still unclear, these characteristics supports the possibility that VP-mediated transduction enhances microbial differentiation and evolution.

Recent developments in genome science have revealed that virus-mediated transduction is not only a phenomenon observed in the laboratory, but contributes to microbial evolution and differentiation.

A comparison of gene sequences between *luxA* and its accessory fluorescent protein together with multilocus housekeeping genes among luminous bacteria of the genus *Aliivibrio*, done by Yoshizawa (35), has made clear that luciferase-related genes in some luminous strains were acquired from the genus *Photobacterium*. These findings suggest the spectral diversity in light emission to be generated by both diversification of the luciferase gene at the genus level and the acquisition of accessory fluorescent proteins. Although HGT would not be responsible for the speciation of luminous bacteria, it would probably be involved in the diversification of emitting light wavelengths shared at the order level. As is predicted from the sequence data (15, 30, 35), HGT must have occurred for the spectral diversity in light emission to have evolved. All the factors necessary for the expression of functional genes in phylogenetically distant species must have been transferred. However, it is impractical to expect the transfer of all functional gene clusters to a
recipient with the aid of conventional gene transfer schemes, since the maximum size of a dsDNA insert in existing gene transfer vectors such as plasmids and coliphage lambda is <25 kbp (plasmid: insert DNA maximum size=15 kbp, phagemid: insert=25 kbp) (1). Thus, an aid capable of transferring a substantial functional gene cluster would be necessary. The possibility of specific particle production having a gene transfer capability was examined in a luminal marine bacterial type strain, *Aliivibrio fischeri* (29). Furthermore, the contribution of the HGT of chromosomal genes to the recipient *Escherichia coli* AB1157 at a high multiplicity of infection (MOI) was examined. The strain was shown to be capable of producing a broad-host gene transfer particle: AfVP, as described above.

**Materials and Methods**

**Bacterial Strains, media, culture conditions, and cell and particle enumeration**

*Aliivibrio fischeri* (NCIMB 1281=ORI No. 194), originally isolated from a light organ of *Euprymna scolopes*, was selected from our laboratory collection. *A. fischeri* was cultured in ZoBell Marine Broth 2216E (Difco, Becton Dickinson, Sparks, MD, USA) and ZoBell Marine Agar 2216E (Difco) at 20°C. The inoculum comprised 3.6×10^6 cells mL^-1, and was incubated at 20°C for 315 h with shaking at 60 rpm. The total volume of the culture was 3.8 L.

Cells were enumerated using samples fixed in 2% (v/v) neutral-buffered formaldehyde (Merck, Darmstadt, Germany) on Anodisc™ 47 membrane filters (pore size 0.2 μm; Advantec Toyo Kaisha, Tokyo, Japan) and stained with SYBR® Gold (Molecular Probe, Invitrogen, Carlsbad, CA, USA), under an epifluorescence microscope (E600; Nikon, Tokyo, Japan) equipped with a super high-pressure mercury lamp with B excitation (450–490 nm) without an ND filter, at a magnification of ×1,000. The number of bacteria was determined from a count exceeding four hundred individuals or 40 eye-fields.

Particles were enumerated by fixing a portion of sample with 10 mM EDTA containing 2.5% (v/v) glutaraldehyde (Wako Pure Chemical Industries, Osaka, Japan) overnight at ambient temperature, and applying it to a carbon and parlodion-coated copper grid (150 mesh; Stork Veco International, Erbeeck, Netherlands) placed in an Epok 812 (Shell Chemicals, Harris Country, TX, USA)-embedded flat-bottomed ultracentrifugation tube and centrifuging at 46,000×g for 90 min at 20°C (2) using a Beckman Preparative Ultracentrifuge L8M with a SW 55.2 Ti rotor (Beckman-Coulter, Fullerton, USA, CA, USA). Grids were stained with 2% (w/v) uranyl acetate (Merck) for 30 s, and then sequentially washed for 15 s with 0.2 μm-filtered deionised and distilled water, and 95% (v/v) ethanol (Wako) twice.

Particle counts were made under a JEM-1200EX electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 80 kV and magnification of ×50,000 as described (2, 7).

**Preparation and purification of virus-like particles**

The culture was spun down at 7,500×g for 40 min at 4°C using a Kubota RT20000 refrigerated centrifuge equipped with a RA-6 rotor (Kubota Medical Appliance Supply, Tokyo, Japan) to separate the cells and the supernatant. The cell pellet was subjected to serial rinsing with TBT [100 mM Tris-HCl, 100 mM NaCl, and 10 mM MgCl₂, pH 7.4; Wako] buffer twice, and centrifugation (8,000×g, 15 min, 4°C), then stored in a deep freezer at −85°C until in situ lysis for Crossfield gel electrophoresis. The rinsing obtained as the supernatant was combined with the above the culture supernatant (total volume, ca. 4 L), filtered with a 0.2-μm membrane filter (CCS-020-E1H, Advantec), and concentrated with Minitan Ultrafiltration Systems using a 100 kDa cut-off membrane (Minitan; Millipore, Bedford, MA, USA), to ca. 15 mL. Free nucleic acid and protein levels were monitored by reading absorbance at 260 nm and 280 nm using a spectrophotometer (Type UV260; Shimadzu, Kyoto, Japan). The concentrated specimen was dialysed against 6 changes of 0.25 L of 10 mM potassium phosphate buffer saline [5 mM MgCl₂, and 200 mM NaCl supplemented phosphate buffer, pH 7.0], then treated with 10 μg mL^-1 each of DNase I and RNase A (Sigma-Aldrich, St. Louis, MO, USA) at 25°C overnight, and finally placed at 80°C for 15 min to inactivate DNase. The treated specimen was spun down at 88,000×g for 40 min at 4°C (Beckman Preparative Ultracentrifuge L8M equipped with a 55.2Ti rotor) to obtain a particle pellet, which was re-suspended in 500 μL of TBT buffer, and dispersed in 5 mL of 35% (w/v) CaCl₂ (Boehringer Mannheim, Mannheim, Germany) in TBT. After CsCl-density gradient equilibrium ultracentrifugation at 178,000×g for 18 h at 4°C, bands containing particles were recovered by a side puncture technique (17). Buoyant densities (ρp) of the bands were determined with an Abbe-refractometer (Atago Seisakusho, Tokyo, Japan), and then specimens were placed in Spectra/Por 4 (molecular weight cut off, MWCO=12,000–14,000; Spectrum Chemicals Mfg, Houston, TX, USA) tubes, and dialysed against 6 changes of 0.25 L of 10 mM phosphate buffer (pH 7.0) at 4°C to remove CsCl. This purified particle is referred to as AfVP, and was used for subsequent experiments.

The nucleic acid and protein contents of the specimens were determined photometrically as above. Protein content was also determined using a Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to its specifications for micro assays.

**Preparation of the recipient, Escherichia coli AB1157, for the gene transfer experiment**

Seed cultures of the recipient *E. coli* AB1157 (F⁻; thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 met-1 proA2 his-4 argE3 rpsL31, ORI No. 194), originally isolated from the National Institute of Genetics, Shizuoka, Japan, were prepared as described previously (10). After confirmation of respective markers, the recipient was grown to ca. 4.0×10^10 CFU mL^-1, and subjected to sequential rinsing with TBT buffer and centrifugation (7,500×g, 40 min, 4°C) twice. The re-suspended cells were re-suspended in 7% (v/v) glycerine (Merck)-containing TBT buffer, dispensed as 1-mL aliquots, immediately frozen in liquid nitrogen, and stored at −85°C prior to use.

**AfVP-mediated gene transfer to Escherichia coli AB1157**

The transduction experiment was carried out as described (5). Briefly, the selection medium was based upon the minimal medium of Davis [MM (%, w/v): 0.2% KH₂PO₄, 0.7% KH₂PO₄, 1.0% (NH₄)₂SO₄, 0.05% sodium citrate (Kyowa Hakko Kogyo, Tokyo, Japan), 0.01% MgSO₄, 0.2% glucose and 1 μg mL⁻¹ thiamine, 1.5% (w/v) agar (Wako, reagent grade)] supplemented with three out of four amino acids [final concentration: 20 μg mL⁻¹ leucine, proline, histidine, arginine]. All media used for the cultivation of *E. coli* AB1157 were supplemented with 100 μg mL⁻¹ of streptomycin (Meiji Seika, Tokyo, Japan).

Recipient cells, *E. coli* AB1157 (F⁻; thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 met-1 proA2 his-4 argE3 rpsL31, ORI No. 194), were mixed with AfVPs to obtain a multiplicity of infection (MOI) of 10, and left undisturbed for 30 min at 30°C. A portion of the mixture withdrawn at 15 min after initiation was fixed with 2.5% (v/v) glutaraldehyde, and particles and cells were counted by EM to determine the practical MOI.

For the examination of lethality, 100 μL of a 10⁻⁵-fold dilution with TBT buffer was plated in triplicate in LB plates (17) with the over layer method with 0.6% TBT agar, and incubated for 1 d at 30°C.

For the examination of gene transfer, the original mixture (100 μL) was plated in the selection medium and incubated for 5 d at 30°C. Two controls were included: 1, recipient cells with TBT buffer instead of AfVPs to determine the spontaneous revertant rate; 2, ultraviolet light (UV)-treated AfVPs and recipient cells. For UV treatment, 400 μL of an AfVP suspension (4.0×10¹⁰ particles) was
placed in a quartz container with a water column thickness of 2 mm, and irradiated with a 12-W UV sterilising lamp (CosmoBio, Tokyo, Japan) for 15 min from a distance of 10 cm. As a reference, the same UV irradiation resulted in a reduction in the plaque-forming ability of coliphage T4 (2×10⁹ PFU mL⁻¹) by seven orders of magnitude (2×10⁶ PFU mL⁻¹).

Colonies generated in the respective selection medium were transferred with a sterile toothpick to all other unselected marker plates, and cultured for 5 d at 30°C, from which results co-ordinated marker transfer was determined. The gene transfer potential of AfVPs could be estimated from this result, since a single infection cannot result in colonies with co-ordinated marker transfer. Furthermore, the gene transfer capability of AfVP can be inferred in relation to the surface exclusion (1) at such a high MOI.

The growth profile of generated colonies was examined in LB broth at 30°C with shaking at 60 rpm.

Electrophoretic analysis of nucleic acid species encapsulated in AfVPs

AfVPs and _A. fischeri_ cells were embedded in 1.0% (w/v) agarose (SeaKem ME; FMC, Philadelphia, PA, USA) gel plugs. An _in situ_ lysis method (8) was adopted, and Crossfield agarose gel electrophoresis was conducted (Crossfield AE-6800; ATTO, Tokyo, Japan). The gel plug was embedded in 1.5% (w/v) agarose, and the gel was run using 0.5×TBE buffer (45 mM Tris-borate, 2 mM EDTA, pH 8.0; Wako) at 14.3 V cm⁻¹ and the gel was run using 0.5×TBE buffer (45 mM Tris-borate, 2 mM EDTA, pH 8.0; Wako) at 14.3 V cm⁻¹; 200 mA; alternate angle, 110°; alternate time, 30 s; at 12°C for 14 h. Nucleic acid species were visualized by staining the gel with a 10,000-fold dilution of SYBR® Gold stock solution in 0.5×TBE buffer at 20°C for 1 h, then illuminated on a Spectroline Trans Illuminator Model TC-365A (Spectronics, Westbury, NY, USA) at 365 nm. Results were recorded with an Olympus E1 single-lens reflex digital camera (Olympus, Tokyo, Japan).

Results

*Aliivibrio fischeri* cell growth and particle production

As shown in Fig. 1, _A. fischeri_ entered a logarithmic phase of growth for up to 12 h immediately after the initiation of culture showing a generation time of ca. 1.84 h, and then entered a stationary phase, in which cell abundance stabilised at ca. 1.2×10¹⁰ cell mL⁻¹ until the end of the culture. Initiation of the production of particles, referred to as AfVPs, by budding (Fig. 1E) deduced from the particle per cell ratio and particle induction frequency (Fig. 1B and D) occurred ca. 15 h after initiation. The numbers of free particles continued to increase up to ca. 50 h after initiation, and then stabilised at 1.2×10¹⁰ particles mL⁻¹. Three sharp peaks (24 h, 100 h, and 200 h) of induction were recorded, while no marked change in the cell population was observed to the end of culture. The average number of mature AfVP particles per cell was ca. 3 (average±SD, 3.0±0.9, n=475; see Fig. 1C). As shown in Fig. 1E, cells with budding particles and other particles were heavily surrounded by mucoidal substances. Budding particles from the cells showed a clear boundary of dense inclusion bodies.

Characteristics of AfVPs observed during purification

From 315 h into the culture of _A. fischeri_, 3.40×10¹⁵ particles in total (100%) were recovered from the broth supernatant by centrifugation (7,800×g, 40 min, 4°C). AfVP production was accompanied by mucoidal substances (Fig. 1E, Dull shape of particles surrounding the cell), a trait observed in marine strains (3, 5), which reduced the rate of recovery using a tangential flow concentration system (0.2 μm-particles>100 kDa). Namely, 1.08×10¹⁵ particles were recovered, corresponding to 39% of the initial number. CsCl density-gradient equilibrium ultracentrifugation resulted in 1.07×10¹⁵ particles (28%). Nucleic acid and protein contents of AfVP in the final specimen were 4.010 mg and 101.764 mg, which corresponded to 29.8% and 35.8% of the initial values, respectively. The ratio of protein per nucleic acid was 24.9, comparable to values reported to date (3, 5–10). Another feature of AfVP is its coagulating behaviour. As often found under EM, AfVPs formed clusters, which could be dispersed by adding EDTA, whose titration point to release entire particles was 6.7 mM.

The buoyant density (ρ₂⁰) of AfVP was between 1.3443 and 1.3980 g cm⁻³ (Fig. 2A). AfVPs had a spherical structure (Fig. 1E), 18.1 to 159.2 nm (95.1% of the overall population) showed a standard probability curve (statistic: D’Agostino-Pearson and Jaque-Bera, significance level, 5%) with a con-
Gene Transfer Particle of A. fischeri 325

The major proportion consisted of particles 40.3 to 76.6 nm in diameter [84.2% of the overall particle population (median±SD, 58.4±11.9 nm, n=528), Fig. 2B], whose estimated DNA content in kbp would vary between 14.6 and 135.7 (Fig. 2C and 3A). The distribution of particle abundance in the sedimentation profile exhibited a similar continual increase in size, an apparent difference from findings concerning the size distribution of particles showing discrete bands, which were composed of several subpopulations (10).

The trend curve showing the relation between the distance of the ultracentrifuge tube from the bottom and buoyant density (Fig. 2A) is given as:

\[
f_{\text{buoyant density}}(d) = -0.0004d^3 + 0.0087d^2 - 0.0978d + 1.5605
\]

\( R^2 = 0.999 \)

Where: \( d \) stands for distance from the bottom in cm. As an extension (Fig. 2C), particle diameter and content as dsDNA would be expressed as the following functions of buoyant density (\( \rho \)).

\[
f_{\text{Particle diameter}}(\rho) = 14,027\rho^3 - 53,695\rho^2 + 67,125\rho - 27,197
\]

\( R^2 = 0.999 \)

\[
f_{\text{Particle DNA content}}(\rho) = -571,110\rho^3 + 2,394,972\rho^2 - 3,347,855\rho + 1,560,453
\]

\( R^2 = 0.999 \)

Results of a nucleic acid type analysis using Crossfield agarose gel electrophoresis (Fig. 3A) gave bands in kbp between 7.4 and 125.8 from \( A. \) fischeri cells and 17.3 and 95.3 from AfVPs as encapsulated molecular mass as dsDNA (Fig. 3B), which accords well with that predicted from the empirical functions (see above, Fig. 2C). The major propor-

Fig. 2. CsCl density gradient equilibrium ultracentrifugation profile of AfVPs produced by \( \text{Aliivibrio fischeri} \) NCIMB1281\textsuperscript{T}.

(A) Superimposed photo placed above shows the result of ultracentrifugation. Trend curve shows relation between ultracentrifuge tube’s distance from bottom and buoyant density (right axis). Relative intensity (left axis) of particle sedimentation profile of the photo as a Gray value obtained by subtracting the background using ImageJ, which is available from the public domain (http://rsbweb.nih.gov/ij/), draws single normal probability distribution. (B) Size distribution of AfVPs showed a standard probability curve (statistic, D’Agostino-Pearson and Jaque-Bera, significance level=5%) with a continual increase in diameter. (C) Relationship between buoyant density versus particle diameter, and encapsulated dsDNA in a particle. Capacity of the particle was obtained at a known packed ratio of dsDNA such as Coliphages T4, lambda and p1 (1, 5). The trend line was drawn by combining other findings for VP (3, 5–6, 8, 10) with the present data. ☐, particle diameter in nm; ●, DNA content of the particle in kbp.

Fig. 3. Molecular type analysis of AfVP encapsulated nucleic acids, and translocation of AfVP content to the recipient cell.

(A) Nucleic acid species encapsulated in AfVPs: Lane 1, Recipient, \( \text{Escherichia coli} \) AB1157, as a negative control; Lane 2, 72 h cultured \( \text{Aliivibrio fischeri} \) cell; Lane 3, AfVP; Lane \( \lambda \), Lambda ladder [48.5 kbp-1.2 Mbp, FMC]; Lane Sc, \( \text{Saccharomyces cerevisiae} \) chromosome [220 kbp-1 Mbp, Nippon Gene, Tokyo, Japan]; and Lane \( \lambda h \), \( \lambda /HindIII \) [Nippon Gene]. (B) Trend line of dsDNA molecular mass for AfVP. Specific migration distance was obtained by comparing with the mass standards on the electrophoretogram using a densitogram created by ImageJ (http://rsbweb.nih.gov/ij/).
tion of AfVPs, obtained as \( p^3 = 1.3560 - 1.3929 \) g cm\(^{-3}\), was used as the material to examine the lethal effect and gene transfer capability.

**AfVP-mediated gene transfer to an auxotrophic**

**Escherichia coli**

The marker *thr-1* was not used because of its high rate of spontaneous reversion (10\(^{-1}\)) (5). Fig. 4A shows an EM image of the AfVP particle content to be under translocation to recipient *E. coli* cytosol. Multiplicity of infection (MOI), the number of particles per cell, was originally designed to be 10, but the observed MOI was actually 140.3. Despite such an extremely high MOI, no lethality in recipient *E. coli* AB1157 cells was observed. Results were expressed as efficiency of plating (EOP), with the number of colonies formed in TBT control plates taken as 100% (average\(\pm SD, 100\% \pm 11\%, n = 9\)). The EOP of UV-treated AfVPs was 136\%\(\pm 14\%\) (n=9). That of untreated AfVPs was 116\%\(\pm 39\%\) (n=9). In other words, AfVP was not lethal to the recipients regardless of UV-treatment. As a result of gene transfer mediated by AfVPs, amino acid deficiencies in the recipient *E. coli* were successfully restored. Transduction frequency is given as fraction of the generated colony number in each selected marker plate in the total applied particles (1.81\(\times 10^5\) particles per plate). Spontaneous reversion frequencies were found to be below the level of detection for the markers employed. No gene transfer was detected in UV-irradiated particles per plate). Spontaneous reversion frequencies were found to be below the level of detection for the markers employed. No gene transfer was detected in UV-irradiated particles. On the contrary, colonies formed in the respective selection medium were: *leu*, 1; *pro*, 27; *his*, 1; *arg*, 48; and MM plate without any amino acid requirements, 29. Result of co-ordinated marker determination revealed that every colony generated in any selected marker plates exhibited growth in any unselected marker plates. Consequently, 129 transductant colonies, whose amino acid auxotrophy was totally restored, were obtained. Transduction frequency for the respective marker is estimated to be: *leu*, 1.5\(\times 10^{-4}\); *pro*, 1.3\(\times 10^{-4}\); *his*, 5.5\(\times 10^{-6}\); *arg*, 2.6\(\times 10^{-4}\); and all amino acid requirements restored, 1.6\(\times 10^{-4}\) CFU per particle.

Particle production from the transductants, referred to as AfV-E-trans, grown in LB broth at 30°C was confirmed. AfV-E-trans acquired budding particle production as shown in Fig. 5B. Maximum growth, corresponding to ca. 415\% of that of the parental *E. coli* recipient, was recorded at 170 h incubation.

**Discussion**

To extend our knowledge of “broad-host range gene transfer particles”, a luminal marine bacterium, *Aliivibrio fischeri*, was examined for its ability to produce a specific particle having a gene transfer capability. This study may initiate effects to elucidate HGT, which is predicted from the sequence data (35).

The oscillating nature of the induction frequency was different from the particle production observed for other microbes taken from various water columns (3–8). The *ca.*

---

**Fig. 4.** Translocation of content from infecting AfVPs to recipient *Escherichia coli* with specific reference to budding particles from *Aliivibrio fischeri* and AfV-E-trans. (A) Diagrammatical representation of particle budding from the cell surface. A discrete boundary can be seen around the budding particle as indicated by an arrow. (B) AfVP budding from *A. fischeri*. (C) Particle budding from AfV-E-trans, a transductant *E. coli* generated with the aid of AfVP-mediated transduction. (D) and (E) Translocation of content from infecting AfVPs to recipient *E. coli*. On transduction, a portion of AfVP and recipient mixture was fixed with 2.5% (v/v) glutaraldehyde at 15 min after initiation and stood undisturbed for 3 h at ambient temperature to determine practical MOI. (D) shows EM image of UV-AfVP infecting the recipient, and (E) is a diagrammatical representation of the translocation of UV-AfVP content into the recipient. Scale bars, 100 nm.

---

**Fig. 5.** Growth profile of AfVP-mediated *Escherichia coli* transductant (AfV-E-trans) with specific reference to parental *E. coli* AB1157 (number per mL\(\pm SD, n = 3\)). (A) AfV-E-trans was cultured in LB medium at 30°C with shaking at 60 rpm. , AfV-E-trans cells mL\(^{-1}\); , AFV-E-trans cells mL\(^{-1}\); , *E. coli* AB1157 cells mL\(^{-1}\). (B) Photo represents budding production of particles from ca. 48 h cultured AfV-E-trans cells. Scale bar, 500 nm.
20% higher induction frequency shown by A. fischeri should have a positive effect on the survival of the strain, since the induction frequency for some marine strains of Alphaproteobacteria fell between 4 and 9% under comparable condition (5). As for the “burst size” of AfVPs, ca. 3 mature particles were observed in an induced cell (Fig. 1C), which is comparable to previous findings (9, 10). As no disrupted particles were observed during the periods corresponding to the decrease in particle per cell ratio (Fig. 1B), it is likely that particles in the broth would again be incorporated into the cells. Similar phenomena were observed for E. coli transductants (10) generated with the aid of particles collected from a geothermal vent at the Toyoha mine (19).

Predicted values for AfVP encapsulated dsDNA were comparable to that obtained from gel electrophoresis (Fig. 2C and 3).

In AfVP-mediated transduction, translocation of AfVP content to recipient E. coli cytosol was observed by EM as shown in Fig. 4D. It is likely that the particle membrane remains outside of the recipient cell, and the string-like structure coated with a proteinaceous material was unravelled as traced in Fig. 4E. Empirical findings make it possible to distinguish infecting particles from budding particles, since the boundary of infecting particle content is unclear. In contrast, budding particles show a clear boundary of its dense content (Fig. 4A–C). An observation done for similar particle production in a cell revealed that the host chromosomal DNA content to recipient (1). In principle, the analysis of genetic co-ordinated marker transfer has arisen from classical genetics of monozygotic crosses (1). Transduction-like gene transfer mediated by AfVPs is somewhat unusual, because additional incorporation in multiple particles seems to promote recipient viability. To estimate the occurrence of transductants with prototrophic reversion of all the four markers adopted, the following approximation was attempted. On the basis of particle volume and coliphage T4 DNA packed ratio (2.34×10^{-24} \text{ L bp}^{-1}), AfVPs would encapsulate linear dsDNA from 1.3 kbp to 902.7 kbp. A major proportion (ca. 84%) of the particle would encapsulate 14.7 kbp to 100.5 kbp, whose diameter varied from 40.3 nm to 76.6 nm. Although AfVPs varied in size, supposing that the major proportion consisted of a median particle size of 58.4 nm, the dsDNA content of the particles is estimated at 44,665 bp. Distance in bp between adopted respective E. coli biochemical marker orthologues on A. fischeri chromosome 1 exceeds the content, which could be encapsulated in one particle: argE3=hisG4=1,478,467; hisG4-proA2=313,679; proA2-leuB6=508,637; and leuB6-argE3=606,441 according to V. fischeri ES114 genome sequence (25). Consequently, it could be hardly expect that a substantial proportion of AfVPs applied would be particles with genetic resources covering all the biochemical markers to be restored in a recipient at a single infection. However, current transduction experiments resulted in transductants with all the adopted markers restored to prototrophy. Therefore, additional incorporation in multiple “infections” of a recipient without any hindrance during transduction is indispensable for generating transductants.

The E. coli transductant successfully acquired the ability to produce particles (Fig. 5). It is notable that the extent of transductant growth was much enhanced in comparison with that of the mother strain, E. coli AB1157. A distinctive difference is apparent for the transducing particles of typical bacteriophages, which contain a maximum of 1% of the host genome in frequency of 10^{-5} to 10^{-7} cells per particle and exhibits nearly 100% lethality (1) in recipient. The “production and incorporation trait” of the particle during the growth certainly endorsed the non-virulence of AfVPs in the transduction experiment. According to the growth profile, the proportion of free particles standing alone was rather scarce in...
the particle population, whereas the major proportion was observed as clustering bodies to make assemblages of more than 100 individuals. When such clustered particles were exposed to EDTA, the proportion of free particle increased as described above. In other words, gene transfer experiment under 10 mM Mg\(^{2+}\) may enhance the chance of the recipient incorporating multiple numbers of AfVPs. Under the circumstances, a high MOI (140) was applied in the gene transfer, which resulted in 129 transductants with all the markers reverted across the phylogenetic order from *Vibrionales* to *Enterobacteriales* with a considerably high transduction frequency.

Concerning HGT in *Vibrionaceae*: Kasai *et al.* (15) deduced from a lux operon sequence analysis that the operon and related gene clusters would have been horizontally transferred from *Aliivibrio* to *Schewanella*. Urbanczyk and colleagues (30) examined lux gene horizontal transfer in *Vibrionaceae*, and concluded that HGT routes based upon results of phylogenetic analyses were not substantially clear, and the contribution of HGT to the speciation would have been inadequate. Despite the prediction, sufficient updated findings are possible to upward revision. Phylogenetic analyses of the lux operon and related gene clusters, done by Yoshizawa (35), elucidated more frequent HGT than that ever considered in *Vibrionaceae*. In relation to this contemporary knowledge, accomplishment of HGT for lux operon and related gene clusters would be an inevitable consequence of a gene transfer scheme capable of huge gene cluster translocation with relaxed restrictions. The current results suggest the horizontal transfer of regulatory gene assemblages to be accomplished by concomitant gene translocation and expression with the aid of non-virulent particles at a high MOI in nature. Together with non-inhibitory effects of additional gene incorporation to the recipient, the trait of AfVP is separate from the conventional virus concept, and is likely to be an apparatus aimed for preservation of the host's chromosomal genes. As we reported, various functional gene clusters such as thermo-resistance was transferred to the E. coli mediated by VP (10). VP-mediated serial transduction supports an indispensable role in microbial differentiation and evolution as mentioned above.

Acknowledgements

This paper is dedicated to the memory of Mari Segawa, who thrived on arduous tasks as a student member of JSME at the beginning of VP-mediated HGT study.

The authors are grateful to S. Yoshizawa, B. Velimirov, R.W. Ridge, M. Nishimura, K. Inoue and 2 anonymous peer reviewers for critical reading, advice, thoughtful comments, and useful suggestions on the manuscript. The authors' research presented here was supported in part by Grant-in-Aid for Scientific Research No. 16310031 from the Japan Society for the Promotion of Science (JSPS), and funded partly by Donations to Encourage Research by Kyowa Hakko Kogyo Ltd and Life Cycle Association Ltd to hxc. Last and certainly not least, thanks are given to the authors' families.

References


