Construction and Use of a Broad-Host-Range Plasmid Expressing the *lamB* Gene for Utilization of Bacteriophage λ Vectors in the Marine Bacterium *Vibrio harveyi*

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Abstract: The remarkable success of *Escherichia coli* as a model organism in molecular genetics was dependent, among other things, on its susceptibility to genetic manipulation. Many versatile and sophisticated genetic tools for molecular biology studies are derived from bacteriophage λ. However, this bacteriophage is specific for *E. coli*, and thus λ-based techniques have been restricted to this bacterium. Plasmids expressing the *E. coli* gene coding for bacteriophage λ receptor were reported previously, and introduction of such plasmids into cells of some other bacteria made them sensitive to phage λ infection. However, we found that these systems were not efficient for *Vibrio harveyi*, one of the most frequently investigated species of marine bacteria. Here we describe construction of a broad-host-range plasmid expressing the *lamB* gene. Introduction of this plasmid to *V. harveyi* cells and expression of *lamB* made this strain susceptible to bacteriophage λ adsorption and λ DNA injection. Foreign genetic material could be introduced into cells of this strain using a cosmid vector.

Key words: *Vibrio harveyi*, genetic manipulations, bacteriophage λ vectors, *Escherichia coli lamB* gene, broad-host-range plasmid cloning vectors.

Introduction

Analysis of cellular processes at the molecular level requires specific tools for genetic manipulation. *Escherichia coli* is the most intensively investigated prokaryotic organism, and current knowledge about many mechanisms of genetic and biochemical processes came from studies on this bacterium. The success of studies on *E. coli* was largely dependent on the availability of genetic tools for DNA transfer between different bacterial strains.

Many versatile and sophisticated vectors are based on bacteriophage λ and its derivatives (Sambrook et al., 1989; Chauthaiwale et al., 1992; Taylor and Węgrzyn, 1998). However, bacteriophage λ infection is specific to *E. coli*; thus other bacteria are not susceptible to genetic manipulation with λ-based constructs. This specificity of bacterio-

Received August 30, 2000; accepted January 30, 2001.
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Table 1. Plasmids

Plasmid	Description	Reference
pAMH62	A pMB1 (ColE1-like) replicon bearing an ampicillin-resistance gene, and the *lamB* gene under control of the *ompR* gene promoter	Harkki and Palva, 1985
pLAFR2	An RK2 replicon bearing also a fragment of the *tra* region and a tetracycline-resistance gene	Friedman et al., 1982
pSKA7	A fusion plasmid bearing all genetic elements of pAMH62 and pLAFR2	This work
pBR328	A pMB1 (ColE1-like) replicon bearing ampicillin-resistance, chloramphenicol-resistance, and tetracycline-resistance genes	Bolivar and Backman, 1979
pHG86	A pMB1 (ColE1-like) replicon bearing the *lacZ* gene and an ampicillin-resistance gene	Giladi et al., 1995
pHG37	A pHG86 derivative bearing a chloramphenicol-resistance gene from pBR328	This work
pSUPTn5pMCS	A p15A (ColE1-like) replicon bearing a transposon Tn*5* with ampicillin-resistance, chloramphenicol-resistance, and trimethoprim-resistance genes	MacKenzie et al., 1995
1AC3	A cosmid bearing the pMB1 (ColE1-like) *origin*, kanamycin-resistance gene, and a fragment of human chromosome 9, the (GT_n) repeat sequence	Kwaitkowski et al., 1992

phage λ host is due to the absence of the phage λ receptor protein (the *lamB* gene product, which apart from being the λ-specific receptor, functions as a maltose porin of the host) in cellular membranes of other bacterial species (Palva et al., 1987). In fact, introduction of a plasmid expressing the *E. coli lamB* gene into cells of some other bacteria (e.g., *Salmonella typhimurium* and *Vibrio cholerae*) made them sensitive to λ infection, though to a lesser extent than *E. coli* (Palva et al., 1981; De Vries et al., 1984; Harkki and Palva, 1984, 1985; Harkki et al., 1986; Salmond et al., 1986).

Vibrio harveyi is a free-living bacterium found in diverse marine environments (Ruby and Morin, 1979; Ruby et al., 1980), which is often used in both basic research and biotechnological applications (for example, see Thomulka and Lange, 1996; Lange and Thomulka, 1997; Czyż et al., 2000a). Because current genetic engineering techniques applicable to *V. harveyi* are significantly restricted relative those applicable to *E. coli*, there is a need for development of additional tools useful in genetic manipulations of this marine bacterium.

In the course of our studies on *V. harveyi*, we tried to use previously described systems for extension of the host range of bacteriophage λ to this bacterium, but with little success (data not shown). Therefore, we aimed to construct a new system allowing us to make *V. harveyi* susceptible to transfer of genetic material mediated by bacteriophage λ vectors.

Material and Methods

Bacterial Strains, Bacteriophages, and Plasmids

Vibrio harveyi wild-type BB7 strain (Belas et al., 1982) was used. *Escherichia coli* wild-type strain MG1655 (Jensen, 1993) was used in most of the control experiments. The MC1061 strain (*hsdR mcrB araD139*, Δ(*araABC-leu*)7679 *lacX74 galU galK rpsL thi*) (Meissner et al., 1987) was used as a host in molecular cloning, the TAP90 strain (*supE44 supF58 hsdR pro leuB thi-1 rpsL lacY1 tonA1 recD1903*::mini-*tet*) (Patterson and Dean, 1987) was employed for phage titration, and the S17-1 strain (*pro hsdR recA*, with integrated plasmid RP4 Tc::Mu-Km::Tn*7*) (Simon et al., 1983), was used as a donor strain in conjunction between *E. coli* and *V. harveyi*.

Bacteriophages λpapa, λ*cIb2* (from our collection), λEMBL4 (*nin*) (Frischauf et al., 1983), λ*cI857S7* (Goldberg and Howe, 1969), and λB299 (Giladi et al., 1995) were used. Phage λB299::*cm* was constructed by in vivo recombination between phage λB299 and plasmid pHG37 (Table 1). The lysate of phage λ*cI857S7* containing radioactively labeled DNA was prepared according to a standard procedure (Arber et al., 1983), but bacteria lysogenic for this phage were grown at 30°C in a minimal medium supplemented with 1% Casamino acids, and [^{3}H]thymidine was added up to 1 μCi/ml at the time of a temperature shift to 43°C. The

radioactivity of the purified phage lysate (after removing an excess of the [^{3}H]thymidine by multiple dialysis) was 3×10^{-5} cpm/pfu.

Plasmids used in this work are listed in Table 1. Plasmid pSKA7 was constructed by ligation of *Eco*RI-digested pAMH62 and pLAFR2 plasmids. For construction of pHG37, a *Bam*HI-*Pst*I fragment of pBR328 (containing a chloramphenicol-resistance gene) was inserted into corresponding sites of pHG86. All molecular cloning procedures were according to Sambrook et al. (1989).

Culture Media and Growth Conditions

The Luria-Bertani (LB) medium (for cultivation of *E. coli* strains) and BOSS medium (for cultivation of *V. harveyi* strains) have already been described by Sambrook et al. (1989) and Klein et al. (1995), respectively. Minimal medium 3 (Węgrzyn and Taylor, 1992) was used, but in the case of *V. harveyi* cultivation, the concentration of NaCl was 3%. The RGMC medium was as described previously (Simon et al., 1983), but NaCl was added to a final concentration of 3% for *V. harveyi* cultivation. Antibiotics were added (when necessary) to the following concentrations: ampicillin up to 50 μg/ml, chloramphenicol up to 35 μg/ml, kanamycin up to 50 μg/ml, and tetracycline up to 12.5 μg/ml. If not indicated otherwise, bacterial strains were cultivated at 30°C.

Bacterial Conjugation

Conjugation between *E. coli* (donor) and *V. harveyi* (recipient) strains was performed according to a previously described method (Simon et al., 1983). The *E. coli* S17-1 strain (containing an integrated plasmid, RP4 Tc::Mu-Km::Tn*7*, which bears genes necessary for mobilization of other plasmids that are not self-transmissible) was transformed with a plasmid to be transferred to *V. harveyi*, and then the plasmid-harboring S17-1 strain was used as a donor in the conjugation with a *V. harveyi* recipient strain.

Maintenance of Plasmid DNA

After growing in the BOSS medium supplemented with appropriate antibiotic, overnight cultures of *V. harveyi* harboring plasmids were diluted 1:100 in a fresh medium devoid of an antibiotic, and cultivation was continued with subsequent dilutions in the fresh medium when the late exponential phase of growth was reached. Samples were withdrawn at indicated times after the first dilution, and bacteria were titrated on BOSS plates with and without appropriate antibiotic. The percentage of antibiotic-resistant cells was calculated, which was considered as a percentage of plasmid-harboring bacteria.

Isolation and Analysis of Outer Membrane Proteins from Bacterial Cells

Outer membrane proteins from *E. coli* and *V. harveyi* cells were isolated according to Nikaido (1994) and Chakrabarti et al. (1996). Briefly, 2.5×10^{10} bacterial cells (from exponentially growing cultures) were harvested by centrifugation (10 minutes, 2000 *g*). The pellet was suspended in 10 ml of a buffer composed of 20 mM Tris-HCl, pH 7.5, and 5 mM phenylmethylsulfonyl fluoride (PMSF). The suspension was sonicated (several 30-second pulses with 30-second intervals) in ice-bath, and then centrifuged (5 minutes, 2000 *g*). The supernatant was ultracentrifuged (1 hour, 100,000 *g*), and the pellet was resuspended in a buffer containing 20 mM HEPES, pH 7.5, and 0.75% Sarkosyl. Following the second ultracentrifugation as described above, the pellet was resuspended in a loading buffer and proteins were separated by electrophoresis in 12.5% SDS polyacrylamide gel. Protein bands were visualized by staining with Coomassie brilliant blue.

Efficiency of Bacteriophage λ Adsorption on Host Cells

Efficiency of bacteriophage λ adsorption on *E. coli* and *V. harveyi* cells was estimated as described previously (Szalewska-Pałasz et al., 1996). Briefly, a phage λ*cI857S7* lysate (titrated on the *E. coli* TAP90 strain at 37°C just prior the experiment) was added to the sample of a bacterial culture to a multiplicity of infection of 1. Following different times of incubation at 30°C, the samples of the mixture were centrifuged (4000 *g* for 1 minute), and the number of unadsorbed phages was estimated by plating the supernatant on the TAP90 strain at 37°C.

Efficiency of Injection of Bacteriophage λ DNA into Host Cells

Efficiency of bacteriophage λ DNA injection into *E. coli* and *V. harveyi* cells was measured by a method similar to that for estimation of bacteriophage λ adsorption on the host cells. However, a lysate of the [^{3}H]thymidine-labeled phage was used, and following addition of the phage lysate to a bacterial culture and 10 minutes of incubation at 30°C, the

mixture was vortexed vigorously for 30 seconds, then centrifuged (4000 *g* for 1 minute), and radioactivities of the supernatant and the pellet were measured in a scintillation counter. The efficiency of phage DNA injection was calculated as a fraction of the total radioactivity found in the pellet of bacterial cells.

In Vitro Packaging of Cosmid DNA into Bacteriophage λ Capsids and Estimation of Efficiency of Transmission of Foreign DNA into Host Cells

Packaging of the 1AC3 cosmid DNA into bacteriophage λ capsids was performed using MaxPlax Lambda Packaging Extract (Epicentre Technologies) and according to the manufacturer's instruction. *Escherichia coli* and *V. harveyi* cells were infected with the phage particles prepared as described above, and serial dilutions of the mixtures were spread on LB or BOSS plates. After overnight incubation at 30°C, the efficiency of transmission of foreign DNA into host cells was estimated from the number of bacterial colonies appearing on plates with kanamycin.

DNA Hybridization

DNA hybridization analysis was performed by a common method described by Sambrook et al. (1989), using a dot-blot apparatus (Bio-Rad, Hercules, Calif.). Chromosomal DNA from bacterial cells was isolated using Genomic DNA Prep Plus kit (A&A Biotechnology). DNA was fixed to a nitrocellulose membrane as described by Sambrook et al. (1989). Cosmid 1AC3 (see Table 1) was used as a template DNA for probe preparation by random-primed incorporation of fluorescein-labeled nucleotides. Hybridization conditions were as described by Śrutkowska et al. (1999). Both preparation of the probe and detection of the signal after hybridization were performed using Random Primer Fluorescein Labeling Kit with Antifluorescein-AP (NEN Life Science Products).

Results

Construction of a Broad-Host-Range Plasmid Expressing the *lamB* Gene

Plasmids bearing the *E. coli lamB* gene had been used previously to make strains of other bacteria sensitive to bacteriophage λ infection (Palva et al., 1981; De Vries et al., 1984; Harkki and Palva, 1984, 1985; Harkki et al., 1986; Salmond et al., 1986). We tried to use such a technique to construct a λ-sensitive strain of marine bacterium *V. harveyi*. Plasmid pAMH62 (Harkki and Palva, 1985) seemed to be the best candidate for sensitizing *V. harveyi* to bacteriophage λ because it contains the *lamB* gene under control of the *E. coli ompR* gene promoter. This promoter was reported to be active in different bacteria, e.g., *S. typhimurium, V. cholerae,* and *Agrobacterium tumefaciens* (Harkki and Palva, 1985). However, our attempts to introduce pAMH62 into a commonly used *V. harveyi* wild-type strain, BB7, by conjugation were not successful (data not shown). This plasmid bears an *origin* of replication region from a ColE1-like plasmid pMB1 (for details, see Harkki and Palva, 1985). Plasmids from this group are unable to replicate in strains outside the enteric bacterial group (Simon et al., 1983). Although it was reported that pAMH62 was introduced into a *V. cholerae* strain by conjugation and maintained in this strain under an antibiotic selection pressure (Harkki et al., 1986), we were not able to repeat this using the *V. harveyi* BB7 (wild-type) strain (data not shown).

We suspected that the inability to obtain a *V. harveyi* BB7 strain bearing pAMH62 might have arisen from impaired replication of the plasmid in this bacterium. We were able to introduce another ColE1-type replicon (bearing the *origin* region from plasmid p15A) into *V. harveyi* BB7, but we found that without an antibiotic selection pressure, this plasmid was relatively poorly maintained in this strain (Figure 1). These results are compatible with the hypothesis presented above.

Because of the problems with obtaining a *V. harveyi* strain containing pAMH62, we constructed an analogous plasmid (called pSKA7) that bears, in addition, the *origin* of replication from a broad-host-range plasmid, RK2. Moreover, pSKA7 contains a fragment of the *tra* region from RK2, allowing its efficient interspecies transmission by conjugation with help of another conjugative plasmid. We were able to introduce pSKA7 into *V. harveyi* BB7 by conjugation (using *E. coli* S17-1 as a donor strain) and found that this plasmid was quite stably maintained in this strain even in the absence of an antibiotic selection pressure (Figure 1).

Expression of the *lamB* Gene in *Vibrio harveyi*

To estimate the level of expression of the *lamB* gene from pSKA7 in *V. harveyi,* and to test whether the *lamB* gene product was properly localized in the outer membrane (this is necessary for efficient adsorption of bacteriophage λ vi-

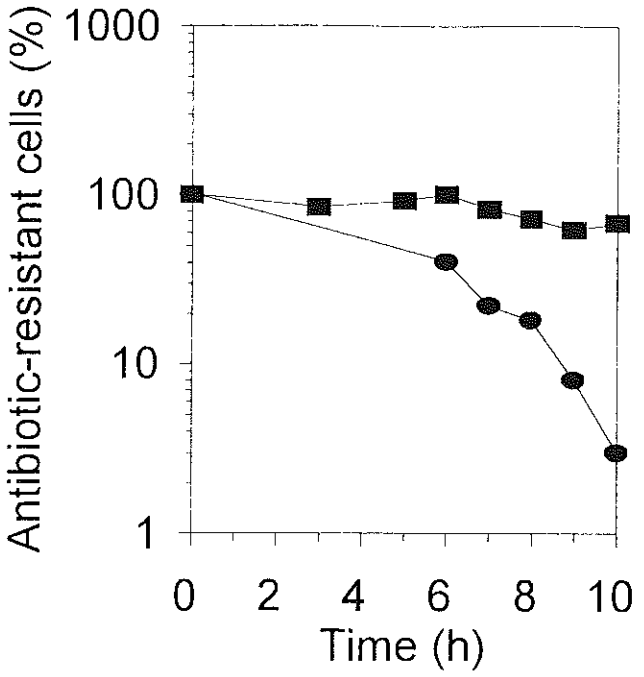

Figure 1. Maintenance of the ColE1-like (p15A-derived plasmid, pSUPTn5pMCS) replicons (circles) and RK2-derived (pSKA7) replicons (squares) in the *V. harveyi* BB7 strain without antibiotic selection pressure.

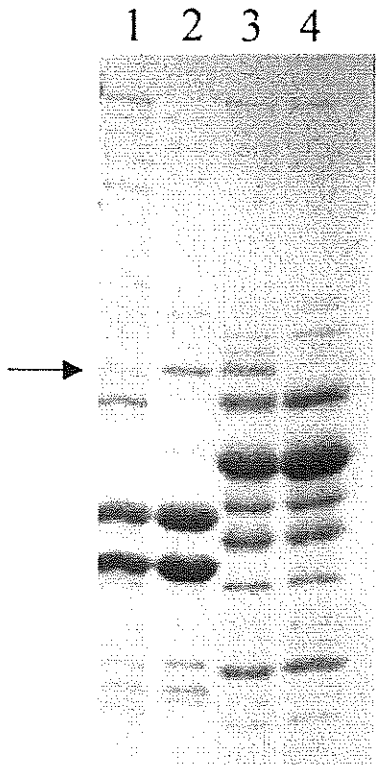

Figure 2. Expression of the *lamB* gene and localization of the gene product in an outer membrane of *E. coli* and *V. harveyi*. Bacterial outer membrane proteins were isolated and separated during sodium dodecylsulfate polyacrylamide gel electrophoresis. Lanes depict material isolated from following bacteria: lane 1, *E. coli* MG1655 growing in minimal medium supplemented with 0.4% glucose; lane 2, *E. coli* MG1655 growing in minimal medium supplemented with 0.4% maltose; lane 3, *V. harveyi* BB7/pSKA7; lane 4, *V. harveyi* BB7. The arrow indicates position of the LamB protein.

rions on the host cell surface), we isolated outer membrane proteins from *V. harveyi* strains bearing pSKA7 and strains devoid of it. In control experiments we isolated outer membrane proteins from the wild-type *E. coli* cells growing in a minimal medium supplemented with either glucose (i.e., with no induction of the *lamB* gene expression) or maltose (i.e., under conditions causing an induction of the *lamB* gene expression). We found that the *V. harveyi* strain bearing pSKA7 produces an outer membrane protein of the size predicted for LamB, which is absent in the wild-type *V. harveyi* (devoid of pSKA7), and which is produced significantly more effectively in *E. coli* cells growing in the presence of maltose relative to those growing without maltose (Figure 2). These results indicate that the LamB protein is produced in the *V. harveyi* BB7/pSKA7 strain, and that expression of the *lamB* gene in this strain is comparable to that found in wild-type *E. coli* cells in the presence of maltose.

Adsorption of Bacteriophage λ on *Vibrio harveyi* Cells Expressing the *lamB* Gene and Phage DNA Injection into Host Cells

Having found that the *V. harveyi* BB7/pSKA7 strain produces the LamB protein effectively, we investigated the efficiency of adsorption of bacteriophage λ on the cells of this strain. We found that contrary to the wild-type *V. harveyi*, which is totally resistant to λ adsorption, this process occurs on the surface of the *V. harveyi* BB7/pSKA7 cells (Figure 3). However, despite the fact that the level of the LamB protein in *V. harveyi* BB7/pSKA7 outer membrane is similar to that in maltose-induced *E. coli* (Figure 2), the kinetics of bacteriophage λ adsorption on the cells of *V. harveyi* BB7/pSKA7 resembles that of *E. coli* grown in the presence of glucose rather than in the presence of maltose (Figure 3).

To estimate efficiency of bacteriophage λ DNA injection into the host cells, we measured the fraction of radioactivity coming from [^{3}H]thymidine-labeled phages that after addition of the bacteriophage λ lysate into a bacterial culture that penetrated inside the host cells. We found that

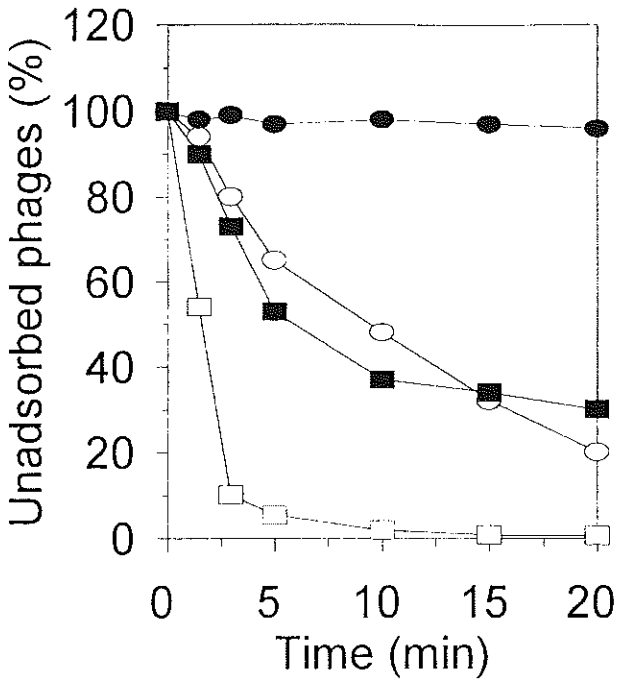

Figure 3. Adsorption of bacteriophage λ*cI857S7* on *E. coli* MG1655 cultivated in minimal medium supplemented with 0.4% glucose (open circles), *E. coli* MG1655 cultivated in minimal medium supplemented with 0.4% maltose (open squares), *V. harveyi* BB7 (solid circles), and *V. harveyi* BB7/pSKA7 (solid squares).

phage λ DNA injection into *V. harveyi* BB7/pSKA7 cells was less efficient than into *E. coli*, but still significant in the light of a lack of detectable transmission of ^{3}H-labeled λ DNA into wild-type *V. harveyi* (Table 2).

Inhibition of Bacteriophage λ Development in *Vibrio harveyi*

Having found that both adsorption of bacteriophage λ on *V. harveyi* BB7/pSKA7 cells and injection of the phage DNA into the host cells are effective, we asked whether this phage would be able to complete its life cycle (either lytic or lysogenic) in the artificial host. We found, however, that neither wild-type λ phage nor its *cIb2*, *nin*, and *vir* derivatives were able to form plaques on *V. harveyi* BB7/pSKA7 (data not shown). The negative results with these combinations of the host and phages were also obtained in the streak test (data not shown). After infection of *V. harveyi* BB7/pSKA7 with bacteriophage λB299::*cm* (bearing a chloramphenicol-resistance gene inserted in the nonessential region of the λ genome), we were not able to obtain chloramphenicol-resistant *V. harveyi* cells, indicating no lysogens. Therefore these results demonstrate that despite effective phage adsorption and DNA injection, neither lytic nor lysogenic development of bacteriophage λ is possible in *V. harveyi*.

Table 2. Injection of λ DNA into Cells of *Escherichia coli* and *Vibrio harveyi* Hosts

Host*	Injected λ DNA† (%)
E. coli MG1655 cultivated in MM-Glu	9.4 ± 1.2
E. coli MG1655 cultivated in MM-Mal	19.8 ± 3.0
V. harveyi BB7 cultivated in MM-Glu	0‡
V. harveyi BB7/pSKA7 cultivated in MM-Glu	3.7 ± 0.9

*Bacteria were cultivated in minimal medium 3 supplemented with 0.4% glucose (MM-Glu) or 0.4% maltose (MM-Mal).
†Bacteriophage λ*cI857S7* DNA injection into host cells was estimated 10 minutes after addition of the phage lysate into bacterial cultures. Average values from 3 experiments ± SD are presented.
‡Efficiency of bacteriophage λ DNA injection was below the level of detection.

Transmission of Foreign DNA into *Vibrio harveyi* Cells Using a Cosmid Vector

To check whether foreign DNA sequences can be transmitted to *V. harveyi* BB7/pSKA7 using bacteriophage λ-derived vectors, we employed a previously described cosmid (1AC3) bearing a ColE1-like *origin* of replication, a kanamycin-resistance gene, and a fragment of the human genome (the $(GT)_n$ repeat sequence. The cosmid DNA was packaged in vitro into λ capsids, and *V. harveyi* BB7/pSKA7 cells were infected with such a construct. Kanamycin-resistant *V. harveyi* colonies were obtained (Table 3), which indicates that the foreign DNA was effectively transmitted into the cells.

We aimed to isolate the cosmid DNA from randomly selected *V. harveyi* kanamycin-resistant cells. The isolation was successful from some clones but not from others (among 27 randomly selected clones, 11 (approx. 40%) did contain the nonintegrated cosmid and 16 (approx. 60%) did not). These results indicated that cosmid DNA can be maintained in *V. harveyi* as a plasmid (though not very efficiently) and, alternatively, foreign DNA sequences provided by a cosmid can integrate into the bacterial chromosome by genetic recombination. To test the latter possibility, we performed dot-blot DNA hybridization experiments using chromosomal DNA isolated from kanamycin-resistant clones lacking a free cosmid DNA, which were

Table 3. Efficiency of Transmission of Foreign DNA into *Escherichia coli* and *Vibrio harveyi* Cells Using a Cosmid Vector

Host	Efficiency of DNA transmission (cfu/μg DNA)*
E. coli MG1655	1.6×10^4
V. harveyi BB7	0†
V. harveyi BB7/pSKA7	1.9×10^3

*DNA of the cosmid 1AC3 was packaged in vitro into λ capsids, and bacterial cultures were infected with the packaging mixture. Serial dilutions were spread on LB or BOSS plates supplemented with kanamycin, and after overnight incubation at 30°C kanamycin-resistant colonies (e.g., transductants containing stably maintained foreign DNA) were counted. The results are expressed as kanamycin-resistant colony forming units (cfu) per 1 μg of the cosmid DNA used for in vitro packaging.
†No kanamycin-resistant cells were observed after overnight incubation.

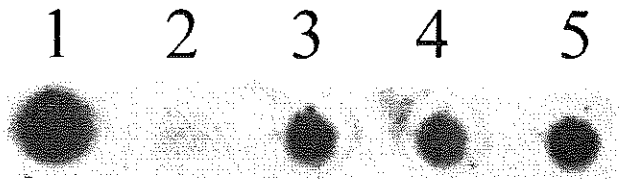

Figure 4. Hybridization (dot-blot) of bacterial chromosomal DNA with a fluorescein-labeled probe constructed using the 1AC3 cosmid DNA as template. The following DNA samples were fixed to a nitrocellulose membrane: dot 1, purified cosmid 1Ac3; dot 2, chromosomal DNA of uninfected *V. harveyi* BB7/pSKA7 cells; dots 3–5, chromosomal DNAs of 3 randomly chosen kanamycin-resistant *V. harveyi* BB7/pSKA7 clones devoid of a free cosmid, selected upon infection of host cells by the 1AC3 cosmid packaged into λ capsids.

obtained after infection of *V. harveyi* BB7/pSKA7 with the 1AC3 cosmid packaged into λ capsids. A fluorescein-labeled DNA prepared by random-primed synthesis on the template of the 1AC3 cosmid was used as a probe. The signal after hybridization was detected in the case of all tested DNA samples isolated from clones described above, in contrast to a control DNA sample isolated from uninfected *V. harveyi* BB7/pSKA7 strain (Figure 4). These results indicate that 1AC3 DNA has been integrated into the bacterial chromosome. We assume that this integration could be due to a recombination process, which should be possible in the presence of $(GT)_n$-like sequences in the *V. harveyi* genome.

Discussion

Vibrio harveyi is one of the commonly used model marine organisms in studies on molecular level, and it is often employed in biotechnological applications (Thomulka and Lange, 1996; Lange and Thomulka, 1997; Czyż et al., 2000a); therefore, new techniques that can make genetic manipulations with this bacterium more efficient should be valuable. This may be especially important because some techniques applicable to other model organisms (e.g., *E. coli*) cannot be used for *V. harveyi*. For example, none of the methods based on a large number of available bacteriophage λ vectors could be used in experiments with *V. harveyi* because this phage is not able to infect cells of this marine bacterium.

The idea of extending the host range of bacteriophage λ to other bacterial species was presented many years ago. In fact, expression of the *lamB* gene, coding for a λ receptor protein, in some bacterial species (e.g., *S. typhimurium, V. cholerae, Klebsiella pneumoniae,* and *Erwinia carotovora*) made them sensitive to bacteriophage λ infection (Palva et al., 1981; De Vries et al., 1984; Harkki and Palva, 1984, 1985; Harkki et al., 1986; Salmond et al., 1986). However, such a procedure was not effective in other bacteria (e.g., *Pseudomonas aeruginosa*) (De Vries et al., 1984; Palva et al., 1987). We tried to sensitize *V. harveyi* BB7 (wild-type) strain to bacteriophage λ by introducing a previously reported plasmid (pAMH62) that contains the *lamB* gene under control of the *ompR* gene promoter, which was shown previously to be active in many bacterial species (Palva et al., 1987). However, we were not able to introduce pAMH62 into *V. harveyi* even using *E. coli* S17-1 strain as a donor containing an integrated helper plasmid able to mobilize other plasmids to be transmitted to recipient cells. Although the available description of pAMH62 did not allow us to determine whether it contains a *mob* region, it was reported previously that this plasmid was successfully transmitted to *V. cholerae* by a conjugation using an *E. coli* donor strain with a helper plasmid (Harkki et al., 1986). Therefore, we assume that pAMH62 is transmissible to different bacteria using a donor *E. coli* strain with *tra* functions, and we suggest that the problems described above arose from restricted replication of the plasmid in *V. harveyi*. This plasmid contains *origin* of replication from a ColE1-like plasmid pMB1.

In our previous study (Czyż et al., 2000b), we were able to introduce another ColE1-like replicon (plasmid pSUPTn5pMCS, which is a p15A derivative) into *V. har-*

veyi. However, we found that without an antibiotic selection pressure this plasmid is relatively poorly maintained in *V. harveyi* BB7. Therefore, it is likely that pAMH62 replication is impaired in *V. harveyi.* It seems, however, that some replication from pMB1 *origin* occurs in this bacterium since after introduction of a cosmid DNA (containing the same type of *origin*) into *V. harveyi* expressing the *lamB* gene from a broad-host-range plasmid, we were able to isolate the cosmid DNA from a fraction of transductants.

Because of the problems with introduction of plasmid pAMH62 into *V. harveyi,* we aimed to construct a broad-host-range plasmid expressing the *lamB* gene. Since other features (apart from the nature of *origin*) of pAMH62 were very useful, we fused this construct to a broad-host-range replicon (RK2-derivative), to obtain plasmid pSKA7. This plasmid was introduced into *V. harveyi* BB7 strain, and analysis of outer membrane proteins of this bacterium revealed that the LamB protein is produced efficiently and localized properly. Both adsorption of bacteriophage λ on *V. harveyi* BB7/pSKA7 and injection of the phage DNA into the host cells were effective, but of lower efficiency relative to *E. coli.* Since the amount of the LamB protein in the outer membrane of *V. harveyi* BB7/pSKA7 was comparable to that in *E. coli,* it seems that other factors apart from the λ receptor, which are different in *V. harveyi* and *E. coli,* are important for the most efficient phage λ adsorption and DNA injection.

Despite effective adsorption and DNA injection, bacteriophage λ was not able to undergo lytic as well as lysogenic development in *V. harveyi* BB7/pSKA7. The use of a phage λ*nin* did not change the results, indicating that defects in λ development are not due simply to a lack of the host-encoded Nus functions in *V. harveyi,* contrary to *S. typhimurium* (Harkki and Palva, 1984). Many host proteins are required for bacteriophage λ development in *E. coli* (Taylor and Węgrzyn, 1998). Thus, if some of them are absent in *V. harveyi,* or analogous or homologous proteins in *E. coli* and *V. harveyi* differ somewhat in their functions, this might block bacteriophage λ development. For example, host heat shock proteins DnaK, DnaJ, GrpE, GroEL, and GroES are absolutely necessary for λ lytic growth (for review, see Polissi et al., 1995; Taylor and Węgrzyn, 1998). In accordance with the above hypothesis, has been demonstrated that functions of some heat shock genes from *V. harveyi* can compensate for deficiency of some functions of homologous *E. coli* heat shock genes, but not for other functions of the same genes (Klein et al., 1998, Kuchanny et al., 1998).

The most important achievement of this work is construction of the *V. harveyi* strain able to accept foreign DNA transmitted by a λ-derived vector (exemplified in this report by a cosmid). Impaired lytic and lysogenic development of bacteriophage λ in *V. harveyi* may be considered as a disadvantage (for instance, it would be difficult to construct a single-copy gene fusion based on bacteriophage λ vectors that can produce single-copy lysogens), but this may be an advantage in some types of experiments. For example, a lack of λ growth in the host cells could provide a simple selection of clones when one would like to replace a wild-type allele in *V. harveyi* chromosome by an allele of this gene inactivated by insertion of an antibiotic-resistance cassette. In the absence of both λ DNA replication and formation of λ prophages, the use of appropriate antibiotic in the medium after infection with a specific bacteriophage λ–based construct should allow for colony formation of only those transductants that possess the antibiotic-resistance gene integrated into the chromosome by a general recombination mechanism. Bacteriophage λ-mediated transposon mutagenesis or cosmid cloning and cosmid-mediated complementation analysis are examples of other possible applications of many currently available bacteriophage λ-based constructs in studies on *V. harveyi.* We hope that the experimental system for genetic manipulations in *V. harveyi,* in which powerful and sophisticated techniques based on vectors and other constructs derived from bacteriophage λ can be employed, will be useful in further basic and applied studies on this marine bacterium.

Acknowledgments

This work was supported by the Polish State Committee for Scientific Research (project grants 6 P04B 022 20 to A.C. and 6 P04G 033 19 to Dr. Hanna Szpilewska, Marine Biology Center of the Polish Academy of Sciences), and by the Marine Biology Center of the Polish Academy of Sciences (task grants 4.2 and 4.4 to G.W.). G.W. acknowledges also financial support from the Foundation for Polish Science (subsidium 14/2000).

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