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Research article

Productive infection and transduction by bacteriophage P1 in the species *Salmonella bongori*

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ABSTRACT

Background: Horizontal gene transfer (HGT) is the most important mechanism in the evolution of new genetic capabilities in bacteria, including specific degradative pathways, virulence factors, and resistance to antibiotics. Among the processes involved in HGT, transduction is noteworthy. This is a mechanism for gene transmission mediated by a bacteriophage that functions both as a reservoir and as a vector of exogenous genes, which remain protected from environmental effects in the bacteriophage's capsid. Within this context, this investigation aimed to evaluate the ability of the generalized transducing bacteriophage P1 to productively infect and transduce in the bacterial species *Salmonella bongori*.
Results: We could establish that a derivative of bacteriophage P1, P1Cm, infects strains of *S. bongori* with frequencies of lysogenization in the order of ~10^{-2} lysogens/UFP.
Through thermal induction, infective viral progeny was obtained, and we could show that P1Cm readily formed plaques on *S. bongori* lawns, a phenomenon thus far not reported for other members of the genus *Salmonella*. Finally, we showed P1Cm-mediated transduction of the model plasmid RP4 at frequencies of ~10^{-7} transductants/donor.
Conclusion: Therefore, bacteriophage P1 can be used as a tool for the genetic manipulation in the species *S. bongori*.
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1. Introduction

Horizontal gene transfer (HGT) by transformation, conjugation, and transduction is a major evolutionary force in bacteria. In particular, transduction, a type of gene transfer mediated by bacteriophages or phages, has the advantage that the non-viral DNA transferred is better protected from environmental injury inside bacteriophage capsids, and its injection into a potential bacterial host is relatively independent of space and time [1]. It is within this context that we present this work on bacteriophage P1.

Bacteriophage P1 is a generalized transducing bacteriophage, originally discovered in *Escherichia coli* [2]. P1 is able to encapsidate up to 100 kb of DNA in phage heads and therefore can transfer linked bacterial chromosomal genes and large plasmids [3]. Consequently, it can contribute to the evolution of P1-infectable bacteria. Actually, P1 has a wide host range. It is able to infect natural isolates of *E. coli* [4] and to transduce genes in *E. coli* and *Shigella dysenteriae* [5], and it interacts with a variety of other enterobacteria [3,6]. In addition, it infects *Myxococcus* [7] and transduces DNA from *E. coli* to *Myxococcus xanthus* [8].

Nevertheless, the genus *Salmonella*, comprising the species *Salmonella enterica* and *Salmonella bongori*, has been generally considered resistant to P1 infection. In fact, Ornellas and Stocker [9], in their classic study, showed that only galE mutants of *S. enterica* serovar Typhimurium, which do not synthetize the LPS O-antigen, are susceptible to P1 infection. Moreover, the serovar Typhimurium did not generate stable P1 lysogens [10], and therefore, the genus *Salmonella* was not considered a suitable host for phage P1.

However, in the landscape of phylogenetical relationships [11] with P1's original host, *E. coli*, there are *S. enterica* serovars more closely related to such host. In this context, León et al. [12] showed that P1 is able to naturally infect the wild-type serovar Choleraesuis and to transduce a multidrug-resistant plasmid despite that no phage P1 plaques were observed in lawns of this bacterial host.

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These results led us to think that *S. bongori*, a species that is closer phylogenetically to *E. coli* [11], should be a more efficient host for phage P1 than the serovar Choleraesuis, and probably as efficient as the original host *E. coli*.

Taken together, our results support the idea presented above.

2. Materials and methods

2.1. Biologicals

Sixteen (16) strains of the species *S. bongori* were used in this study. They were generously donated, from his collection, by Dr. Roy Curtiss III, then at Biodesign Institute, Arizona State University.

The bacteriophage used was P1Cmlclr100 [13], here on referred to as P1Cm. This P1 derivative harbors transposon Tn9, which confers chloramphenicol (Cm) resistance to lysogens. Furthermore, it has a temperature-sensitive (ts) repressor that allows induction of the lytic cycle at temperatures above 34°C.

Additional bacterial strains are indicated in the text. Bacteriological media or components thereof were purchased from Oxoid (Basingstoke, UK). Antibiotics and reagents were obtained from Sigma (St. Louis, MO).

2.2. P1Cm lysogenization and preparation of lysates

Procedures were essentially as described by Miller [13].

S. bongori strains were infected with P1Cm and Cm-resistant lysogenic colonies were recovered after incubation at 30°C. Lysates were prepared from P1Cm lysogenic strains by thermal induction of the respective cultures.

2.3. Lysogen stability

P1Cm lysogens were grown in LB broth at 30°C and serially transferred to fresh medium (1:10 dilution) for up to 75 generations. The last culture was diluted appropriately and plated on LB agar. After incubation at 30°C for 48 h, a minimum of 100 colonies were tested for Cm resistance and thermosensitivity at 42°C.

2.4. Transduction

P1Cm lysates were prepared from suitable donor strains [13] carrying plasmid RP4 [14]. The receptor strain was grown overnight at 37°C with aeration. Cells were pelleted by centrifugation, and after discarding the supernatant, they were suspended in an equivalent volume of MC buffer (100 mM $MgSO_4$, 5 mM $CaCl_2$). Then 0.1 of the cell suspension was mixed with 0.1 mL of the donor lysate and the mixture incubated for 30 min at 30°C; 0.2 mL of 1 M sodium citrate was added together with 3 mL of molten (45°C) LB soft agar (0.65%).

The whole mixture was poured onto selective LB agar plates containing kanamycin (Km, 50 μg/mL), ampicillin (Ap, 50 μg/mL), tetracycline (Tc, 20 μg/mL), and nalidixic acid (Nal, 50 μg/mL) to allow selection of plasmid RP4 transductants of *E. coli* C by incubation at 42°C for 24 to 48 h. This also prevents lysogenization of transductants.

2.5. Molecular techniques

These techniques were performed essentially as described by Sambrook and Russell [15].

PCR amplifications of the P1 c region and the RP4 *trfA* gene were achieved using the following primers (IDT): *c1.100* Forward (5′-TTGC GGCTGCGCGTAACTGA-3′) and c1.100 Reverse (5′-TCGCCCGGTTGTCG TTCTGC-3′) for P1 and Forward (5′-TTCACCSTTCTACGAGMTKTGCCAG GAC-3′) and trfA 1013 Reverse (5′-GWCAGCTTGCGGTACTTCTCCCA-3′) [14] for RP4. Amplified DNA fragments were analyzed by agarose (2%) gel electrophoresis.

Table 1
Frequencies of lysogenization in *S. bongori* strains (N = 3).

Bacterial strains	Frequency of lysogenization (lysogens/PFU)
χ 9610	1.2×10^{-1}
χ 9611	1.6×10^{-1}
χ 9612	2×10^{-1}
χ 9613	NI
χ 9614	6×10^{-2}
χ 9615	3×10^{-2}
χ 9616	5×10^{-2}
χ 9617	2.4×10^{-1}
χ 9618	3×10^{-2}
χ 9619	1×10^{-2}
χ 9620	1.6×10^{-1}
χ 9621	9×10^{-2}
χ 9622	NI
χ 9623	2×10^{-2}
χ 9624	1.2×10^{-1}
χ 9625	NI
E. coli	2.8×10^{-1}

3. Results

Our first goal was to determine infection and lysogenization of *S. bongori* by phage P1Cm.

Table 1 shows that 13/16 strains of *S. bongori* could be lysogenized and four of them at frequencies of 10^{-2} to 10^{-1}.

All strains infected could grow at 30°C in the presence of Cm and were unable to grow at 42°C. The presence of P1Cm was checked by PCR amplification of an internal segment of the *c1.100* gene corresponding to the P1Cm's ts repressor (Fig. 1a).

Furthermore, P1 lysogens were stable for at least 75 generations. All clones examined were not able to grow at 42°C but preserved resistance to Cm when grown at 30°C.

Successful lysogenization should also entail P1Cm production by the stable lysogens, and in fact, upon thermal induction of the four strains that exhibited the highest frequencies of lysogenization, we obtained P1Cm lysates with titers comparable to those yielded by an *E. coli* lysogen. Particularly, in the case of the strain χ 9617, the ratio of *S. bongori*/*E. coli* P1Cm yield was 1.4.

In addition, when the χ 9617 P1Cm lysate was used to infect the three strains that were not initially lysogenized by P1Cm, lysogens were readily obtained.

However, a definitive criterion of bacteriophage infection and in the case of P1Cm of an eventual transducing capacity is the ability to form plaques in the corresponding bacterial host. Fig. 1b shows a P1Cm macroplaque on *S. bongori* at 42°C.

A logical extension of the observations above is that P1Cm should be able to transduce genetic material from a *S. bongori* lysogenic host. To test this, we used a *S. bongori* χ 9617 derivative containing the model plasmid RP4 coding for resistance to Ap, Tc, and Km [14]. This χ 9617 derivative was lysogenized with P1Cm, and donor lysates were prepared by thermal induction to test RP4 transduction to an *E. coli* receptor.

Fig. 2a shows the presence of the plasmid RP4 in the χ 9617 derivative by amplification of the trfA gene in RP4, and Table 2 shows the results of RP4 transduction by P1Cm.

Transductants were checked phenotypically and by *trfA* detection (Fig. 2b).

In addition, we constructed a Nal-resistant derivative of the *S. bongori* strain χ 9611 containing RP4 and lysogenic for P1Cm. From such derivative, we could detect RP4 transduction to the parental strain, which became resistant to Ap, Tc, and Km but remained Nal sensitive, as expected.

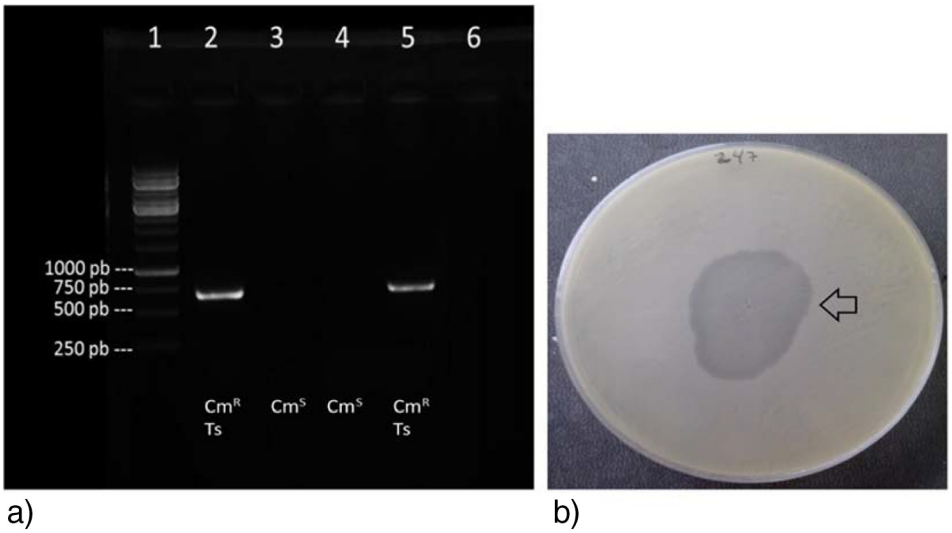

Fig. 1. (a) Amplification of the c1.100 segment of the ts repressor gene in P1Cm. (1): Molecular mass marker 1 kb; (2): *E. coli* P1Cm lysogen; (3): non-lysogen *E. coli*; (4): *Salmonella bongori* χ 9617; (5): *S. bongori* χ 9617 P1Cm lysogen; (6): PCR negative control. (b) P1Cm lysis macroplaque on *S. bongori* χ 9617 at 42°C.

4. Discussion

Regarding the natural infection and stable lysogenization of *S. bongori* by phage P1Cm, our results clearly show that the process is very similar to that observed in P1's original host, *E. coli*. Indeed, plaque formation and P1Cm yields are essentially the same. We speculate that the O region, which is the core region of LPS, the site for P1 attachment, is similar in both *E. coli* and *S. bongori* in terms of not hindering the P1–bacterial cell interaction.

On the other hand, when the process is compared to that in *S. enterica* serovar Choleraesuis [12], results with *S. bongori* as the host show an improvement in terms of frequency of lysogenization, which is approximately two orders of magnitude higher in *S. bongori* than in the serovar Choleraesuis. In addition, as in the serovar Choleraesuis, lysogens were stable for at least 75 generations. In addition, plaque formation in *S. bongori* is a novel feature not observed in the serovar Choleraesuis or in any other *S. enterica* serovar tested thus far [12]. Therefore, it is safe to conclude that the life cycle of phage P1 in *S. bongori* is more efficient than that in the serovar Choleraesuis. This is probably due to the fact that *S. bongori* is phylogenetically closer to *E. coli* than to the serovar Choleraesuis [11]. This line of reasoning would also explain the poor performance of the serovar Typhimurium in its interaction with P1 [10].

Our results also show that P1Cm is fully functional regarding its capacity to out-transduce genetic material from its *S. bongori* host. In fact, we observed transduction of the model plasmid RP4 to *E. coli* and *S. bongori* at a frequency similar to that registered in the serovar Choleraesuis for transduction of the RP4 plasmid derivative pLM2 [12]. Furthermore, plasmid transduction by P1 has been shown to occur at similar frequencies in *E. coli* [13,16].

In nature, *S. bongori* is found to be associated with the intestinal tract of birds and reptiles, sharing habitat with *E. coli* and a variety of

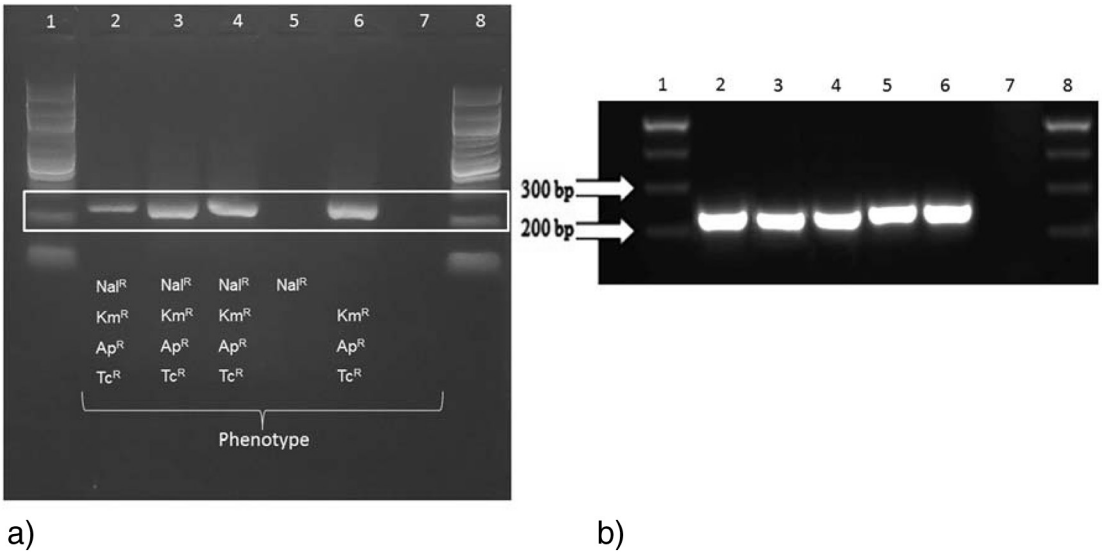

Fig. 2. Molecular determinations associated with P1Cm-mediated transduction of plasmid RP4: (a) Amplification of the *trfA* gene in plasmid RP4. (1 and 8): Molecular mass markers (100 bp); (2, 3, and 4): *S. bongori* RP4 transconjugants; (5): *S. bongori* χ 9617 without RP4; (6): *E. coli* containing RP4; (7): PCR negative control. (b) Detection of the RP4 *trfA* gene after P1Cm-mediated transduction. (1 and 8): Molecular mass markers (100 bp); (2 and 3): *E. coli* conjugative donor strains containing RP4; (4 and 5): *S. bongori* transconjugant, donor strains containing RP4; (6): *E. coli* RP4 transductant; (7): *E. coli C*.

Table 2
Determination of transduction frequency.

Lysate titers (PFU/mL) χ 9617 (RP4·P1Cm)	No. of transductants *E. coli* C	Freq. of transductants/ recipient
1.- 1.57×10^{7}	82	5.22×10^{-6}
2.- 1.47×10^{7}	123	8.36×10^{-6}
3.- 3.0×10^{7}	108	3.6×10^{-6}

other Gram-negative bacteria, some of which are susceptible to P1 infection [16,17]. Therefore, one could envisage that P1 or P1-like phage might participate in the network of genetic interactions among related bacterial genera [18], in which *S. bongori* could be a protagonist in terms of donating or acquiring genetic information, particularly plasmids coding for drug resistance, an issue of concern, as *S. bongori* has already been defined as a pathogen in humans [19, 20] and could be thus potentiated as an emergent bacterial infectious agent. Additionally, bacteriophage P1 could be used as an aid for the genetic modulation of *S. bongori*.

In summary, we have shown natural infection, lysogenization, and transduction by phage P1 in *S. bongori* and the potential of such viral agent to promote evolution in this newly defined bacterial host and to assist in its genetic manipulation.

Therefore, bacteriophage P1 or other bacteriophages with similar features could play a role in the evolution of *S. bongori* and its potentiation as an emergent pathogen.

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Conflict of interest

Authors have no conflict of interest.

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