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Characterization of pES213, a small mobilizable plasmid from *Vibrio fischeri*

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Abstract

Most *Vibrio fischeri* strains isolated from the *Euprymna scolopes* light organ carry plasmids, often including both a large (>40 kb) plasmid, and one or more small (<12 kb) plasmids. The large plasmids share homology with pES100, which is the lone plasmid in *V. fischeri* type strain ES114. pES100 appears to encode a conjugative system similar to that on plasmid R721. The small plasmids lack extensive similarity to pES100, but they almost always occur in cells that also harbor a large plasmid resembling pES100. We found that many or all of these small plasmids share homology with pES213, a plasmid in strain ES213. We determined the 5501-bp pES213 sequence and generated selectable antibiotic resistance encoding pES213 derivatives, which enabled us to examine replication, retention, and transfer in *V. fischeri*. An 863-bp fragment of pES213 with features characteristic of θ-type replicons conferred replication without requiring any pES213 open reading frame (ORF). We estimated that pES213 derivatives were maintained at 9.4 copies per genome, which corresponds well with a model of random plasmid segregation to daughter cells and the $\sim 10^{-4}$ per generation frequency of plasmid loss. pES213 derivatives mobilized between *V. fischeri* strains at frequencies up to $\sim 10^{-4}$ in culture and in the host, apparently by employing the pES100 conjugative apparatus. pES213 carries two homologs of the putative pES100 origin of transfer (*oriT*), and *V. fischeri* strains lacking the pES100 conjugative relaxase, including a relaxase mutant, failed to serve as donors for transmission of pES213 derivatives. In other systems, genes directing conjugative transfer can function in *trans* to *oriT*, so it was noteworthy that ORFs adjacent to *oriT*, VFB51 in pES100 and *traYZ* in pES213, enhanced transfer 100- to 1000-fold when provided in *cis*. We also identified and disrupted the *V. fischeri recA* gene. RecA was not required for stable pES213 replication but surprisingly was required in donors for efficient transfer of pES213 derivatives. These studies provide an explanation for the prevalence and co-occurrence of pES100- and pES213-type plasmids, illuminate novel elements of pES213 mobilization, and provide the foundation for new genetic tools in *V. fischeri*.

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1. Introduction

The marine γ-proteobacterium *Vibrio fischeri* serves as an important model for studies of bioluminescence, quorum-sensing gene regulation, and beneficial animal–bacteria interactions. Each of these fields has enhanced our understanding of *V. fischeri* genetics and made use of genetic tools in *V. fischeri*. In particular, the recent development of the light organ symbiosis between *V. fischeri* and the Hawaiian bobtail squid, *Euprymna scolopes*, as a model animal–bacteria mutualism has driven interest in *V. fischeri* genetics, because unlike bioluminescence and quorum-sensing phenomena this complex symbiosis cannot be effectively studied using a subset of *V. fischeri* genes cloned in *Escherichia coli*. This interest in the genetics of symbiotic *V. fischeri* prompted the recent genome sequencing of *V. fischeri* strain ES114, a wild-type isolate from *E. scolopes*. ES114 was chosen as a type strain partly for its genetic simplicity, in that it lacks small plasmids and contains only a single large (45.8-kb) plasmid, pES100. As a result, the genome project did not encompass small *V. fischeri* plasmids.

Vibrio fischeri symbionts of *E. scolopes* often harbor plasmids. Boettcher and Ruby (1994) found that 56% of *V. fischeri* isolates from *E. scolopes* carried plasmids, and of these 15% carried a large plasmid with homology to pES100, 4% carried small (<12-kb) plasmids that lacked significant homology to pES100, and 81% harbored both a large plasmid similar to pES100 and one or more small plasmids. Although this frequent co-occurrence of distinct large and small plasmid types suggested a connection between them, no functional interrelationship was determined. However, conjugation may play an important role in plasmid distribution among *E. scolopes* isolates, and the light organ, which is densely colonized by closely related bacteria, appears especially conducive to plasmid transfer. Only *V. fischeri* colonizes this tissue, usually as a mix of strains, and hundreds of millions of cells are packed at densities of $10^{10}\,ml^{-1}$ (Ruby, 1996). Although plasmid exchange in this natural setting seems likely, it has not been documented.

pES100 is large enough to encode a complete conjugative apparatus, but the small *V. fischeri* plasmids must either utilize a remarkably compact conjugative system or, more likely, co-opt factors from pES100 or one of the two *V. fischeri* chromosomes to mobilize. In other described systems, small plasmids need only carry an origin of transfer (*oriT*) in *cis* if the cognate relaxase is provided in *trans* in combination with other DNA-processing proteins and a type-IV secretion system (Burns, 2003; Cascales and Christie, 2004; Francia et al., 2004; Frost et al., 1994; Pansegrau et al., 1994; Pansegrau and Lanka, 1996). However, even if these *V. fischeri* plasmids are transmissible it is not known whether the conjugative system belongs to a well-characterized family or one of the groups lacking a well-described representative (Francia et al., 2004). Therefore, the mechanisms of *V. fischeri* plasmid transfer could include novel elements.

Also unknown are the mechanisms by which these *V. fischeri* plasmids replicate. θ-type replicons are prevalent in the γ-proteobacteria, but among these plasmids there is considerable diversity in the specific composition of the replication origin, *oriV* (Del Solar et al., 1998). Delineating the requirements for replication of *V. fischeri* plasmids will add to our understanding of plasmid replication strategies, and it will constitute an important first step in developing useful new vectors. Native *V. fischeri* plasmids have not been exploited as genetic tools in this bacterium despite the growing interest in *V. fischeri* as a model system and the shortcomings of plasmids currently available.

In this study, we sequenced and characterized *V. fischeri* plasmid pES213. This plasmid was found in strain ES213, an isolate from *E. scolopes* that contains both a large plasmid with significant homology to pES100 and multiple smaller plasmids (Boettcher and Ruby, 1994). Our results illuminate the mechanisms by which pES213 is replicated, maintained, and mobilized in *V. fischeri*. Our data suggest that pES100 is a conjugative