Nucleotide Sequence of IS492, a Novel Insertion Sequence Causing Variation in Extracellular Polysaccharide Production in the Marine Bacterium Pseudomonas atlantica

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The complete nucleotide sequence of insertion element IS492, which causes reversible inactivation of extracellular polysaccharide production in the marine bacterium Pseudomonas atlantica, is presented. Insertion of IS492 results in the EPS strain and excision results in restoration of EPS. DNA sequencing of the site of insertion in the eps locus showed that insertion of IS492 generates a 5-base-pair repeat and that its excision is precise. IS492 is 1,202 nucleotides in length and contains one large open reading frame encoding a protein of 318 amino acids, a candidate for transposition function. No similarity between IS492 and other transposable elements has been found. Unlike the situation with other insertion sequences, no direct or inverted repeats exist at the termini of IS492.

Variable phenotypes in clonal populations of bacteria are often the result of reversible DNA rearrangements. Examples include the inversion of DNA that regulates flagellar phase variation in Salmonella typhimurium, recombination between silent and expression plin loci in Neisseria gonorrhoeae, and IS1-like transposition regulation in Citrobacter Vi antigen expression in Escherichia coli (12, 14, 15). We have recently described an insertion element that regulates variation in extracellular polysaccharide production in the marine bacterium Pseudomonas atlantica (3). Mucoi EPS cells give rise to nonmucoid EPS cells which have a characteristic crenated colony morphology and which can switch back to the EPS phenotype. The EPS phenotype results from the site-specific insertion of a 1.2-kilobase-pair (kbp) DNA segment into an eps gene, and switching back to the EPS phenotype results from excision of the 1.2-kbp segment (3). IS492 insertions in numerous EPS variants have been mapped by restriction fragment analysis, and these insertions appear to have occurred at identical locations in the eps locus (3). We conclude that insertion is occurring at one site or several sites in a very small region of about 50 bp. The relationship between the EPS phenotype and the structure of the eps locus is shown in Fig. 1. Antigenic variability is believed to be an important mechanism for immunological evasion in infection by pathogenic bacteria, and the generation of diversity of particular cell surface properties could be important to the survival of bacteria such as P. atlantica in a changing marine environment. For example, EPS cells could be more adhesive to surfaces (favoring microcolony development), while EPS cells might be adapted for a planktonic existence. This paper describes the cloning and DNA sequence determination of the P. atlantica insertion element and the region of the eps gene into which it integrates. The DNA sequence analysis indicates a unique mobile genetic element which we named IS492.

DNA sequencing. Restriction fragments containing the eps locus from the parental EPS strain, from an EPS variant (descendant of the parental strain), and from an EPS variant (descendant of the EPS strain) were subcloned into M13mp8 for DNA sequencing. The subclones in M13 thus represent sequences from a direct lineage of variants, EPS to EPS to EPS. Specifically, the 2.0-kbp HindIII fragment with the left portion of eps (as diagrammed in Fig. 1) was subcloned into M13 from a recombinant plasmid (pDB200) which contains the undisturbed eps gene from the parental mucoid strain (3). This HindIII fragment contains the region of the eps gene into which IS492 inserts (Fig. 1A). The 1.9-kbp HindIII fragment, containing the left portion of eps and IS492, and the 1.2-kbp HindIII fragment, containing the right portion of IS492 and 126 bp of eps, were subcloned from a recombinant plasmid (pDB440) which contains the eps locus disrupted by IS492 insertion (Fig. 1B). Finally, the 2.0-kbp HindIII fragment was subcloned from a recombinant plasmid (pDB4401) isolated from an EPS strain which arose from the EPS variant that contained pDB440. This fragment carries the eps locus from which IS492 had excised (Fig. 1C). The derivation of the recombinant eps plasmids pDB200, pDB440, and pDB4401 has previously been described (3). Sequencing was performed by the Sanger dye-oxym method (13) with [*S]dATP (New England Nuclear Corp., Boston, Mass.) and Sequenase DNA polymerase (United States Biochemical Corp.). Subsequent sequencing steps were as previously described (2). Initial DNA sequencing used the M13 universal primer, but DNA synthesis farther along the template was initiated with oligonucleotide primers designed from the previous DNA sequence. Both strands of IS492, as well as portions of the eps locus from the three strains in the EPS lineage, were sequenced.

Insertion and excision of IS492. The DNA sequence of IS492 and the region of the eps gene flanking its site of insertion is shown in Fig. 2. IS492 is 1,202 nucleotides in length. An unusual feature of this element is the lack of either direct or inverted repeats at its termini. Of the first 25 nucleotides from both ends of IS492, only 8 nucleotides and 7 nucleotides are matched for inverted- and direct-repeat alignments, respectively. A single open reading frame more than 200 bp long was found after examination of both strands

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FIG. 1. Correlation between colony morphology and eps structure in *P. atlantica*. Photographs of *P. atlantica* colonies are shown on the left (magnification, ×10), while a physical map of the eps locus is shown on the right. (A) Mucoid colony. (B) Crenated colony. The right side shows an IS492 insertion into the eps locus. (C) Crenated colony containing two mucoid sectors which resulted from the excision of IS492. H, HindIII; Bg, BglII.
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In the open reading frame encoding a protein of 318 amino acids.

of IS492: it extended from position 228 to 1181, encoding a 318-amino-acid protein. No canonical sigma 70 promoter sequence is evident upstream of this 318-amino-acid open reading frame (7). The sequence of eps DNA before insertion, after insertion, and after excision of IS492 was compared. Insertion results in a 5-bp target duplication, which consists of the sequence ACAAG. Similar sequence comparison of eps DNA which has lost IS492 revealed that IS492 excision was precise, removing the entire insertion sequence as well as one of the target site duplications.

On the basis of these findings, the element causing EPS variation in *P. atlantica* appears to be an insertion sequence, and we have designated it IS492. The termini of IS492 were defined by sequencing a portion of the eps locus with and without the presence of IS492. The size of IS492 was 1.203 kilobases (kb). Furthermore, it contains no *HpaII* sites and has one asymmetric *HindIII* site (3). The placement of a variety of other restriction enzyme cleavage sites within the sequence of IS492 also confirms restriction mapping experiments (data not shown). IS492 has previously been shown to be present in multiple copies in the *P. atlantica* genome and to undergo RecA-independent transposition (3). The nucleotide sequence data presented here provide additional indications that IS492 is indeed an insertion sequence. Insertion of IS492 into the *eps* locus generates a 5-bp target duplication, as does insertion of IS2, Tn3, or bacteriophage Mu (1, 6, 11). Like many other insertion sequence elements, IS492 also encodes one long open reading frame between 300 and 400 amino acids long (8). The Chou-Fasman algorithm (4) applied to this protein predicts a cytoplasmic cellular localization, since there are no membrane-spanning domains, and the predicted isoelectric point of this protein is 10.89, which is consistent with its expected affinity for binding nucleic acids.

A search for similarity between IS492 and sequences present in the National Institutes of Health-GenBank or European Molecular Biology Laboratory data bases was made with the wordsearch program of the University of Wisconsin GCG package (5), but no striking similarities were revealed. In addition, no significant similarity between the IS492 hypothetical protein and protein sequences present in the National Biomedical Research Foundation data base was found. One unusual characteristic of IS492 is that it lacks inverted or direct repeats at its termini. This absence of terminal repeats has been observed only in the *Staphylococcus* transposon Tn534 and in phage Mu (9, 10). IS492 is the transposable element isolated from a marine eubacterium, so its apparent novelty could be due to the limited attention given to this large and diverse group of organisms.

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