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Nucleotide Sequence of IS*492*, 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 IS*492*, which causes reversible inactivation of extracellular polysaccharide production in the marine bacterium *Pseudomonas atlantica*, is presented. Insertion of IS*492* results in the EPS$^-$ phenotype, and excision results in restoration of EPS$^+$. DNA sequencing of the site of insertion in the *eps* locus showed that insertion of IS*492* generates a 5-base-pair repeat and that its excision is precise. IS*492* 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 IS*492* and other transposable elements has been found. Unlike the situation with other insertion sequences, no direct or inverted repeats exist at the termini of IS*492*.

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 pilin loci in *Neisseria gonorrhoeae*, and IS*1*-like transposition regulation of *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). Mucoid 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). IS*492* 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 IS*492*.

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$^-$ variant) 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 *Hin*dIII fragment with the left portion of *eps* (as diagrammed in Fig. 1) was subcloned into M13 from a recombinant plasmid (pDB200) which contains the undisrupted *eps* gene from the parental mucoid strain (3). This *Hin*dIII fragment contains the region of the *eps* gene into which IS*492* inserts (Fig. 1A). The 1.9-kbp *Hin*dIII fragment, containing the left portion of *eps* and IS*492*, and the 1.2-kbp *Hin*dIII fragment, containing the right portion of IS*492* and 126 bp of *eps*, were subcloned from a recombinant plasmid (pDB440) which contains the *eps* locus disrupted by IS*492* insertion (Fig. 1B). Finally, the 2.0-kbp *Hin*dIII fragment was subcloned from a recombinant plasmid (pDB4401) isolated from an EPS$^+$ variant which arose from the EPS$^-$ variant that contained pDB440. This fragment carries the *eps* locus from which IS*492* 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 dideoxy method (13) with [^{35}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 IS*492*, as well as portions of the *eps* locus from the three strains in the EPS lineage, were sequenced.

Insertion and excision of IS*492*. The DNA sequence of IS*492* and the region of the *eps* gene flanking its site of insertion is shown in Fig. 2. IS*492* 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 IS*492*, 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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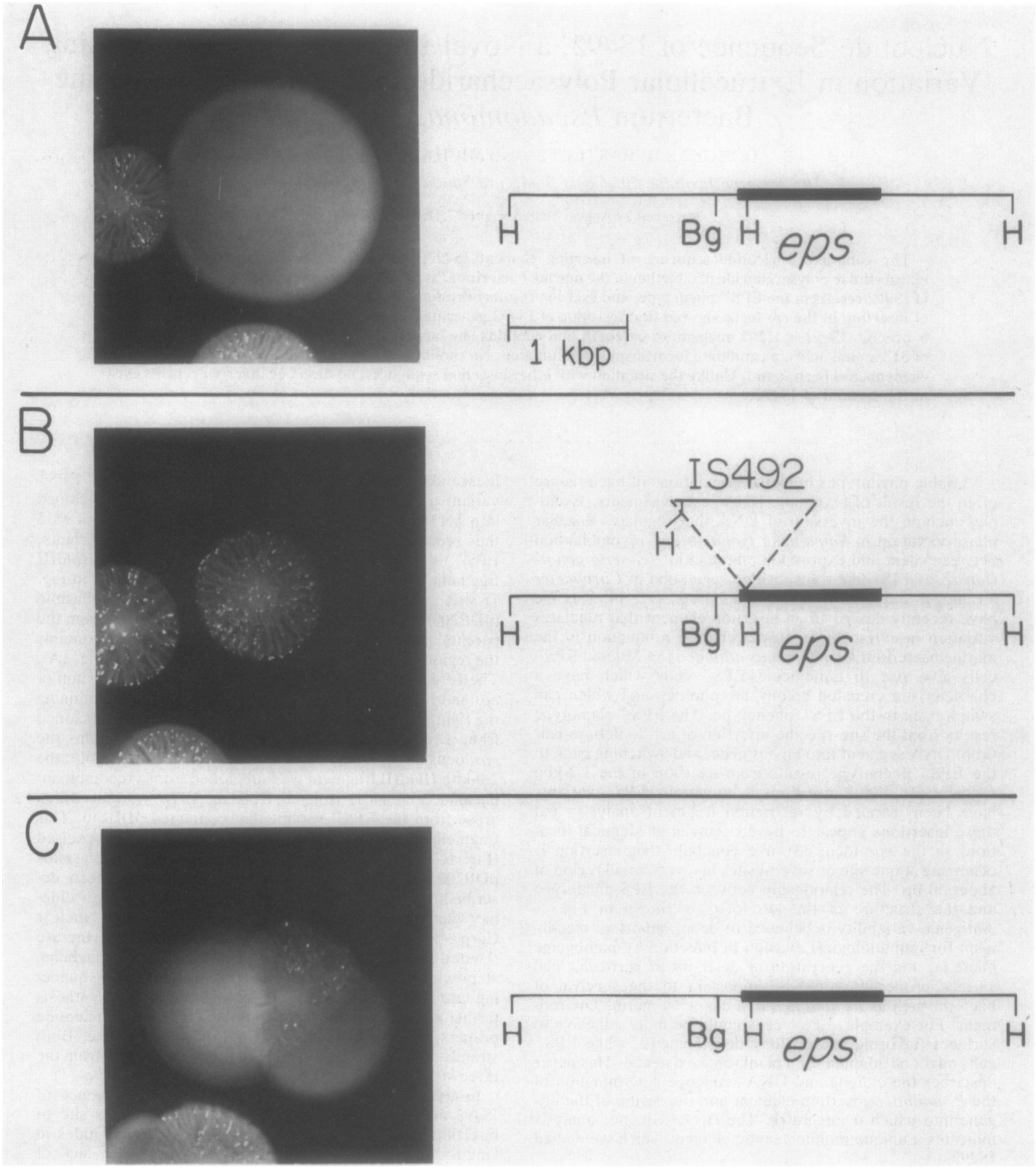

FIG. 1. Correlation between colony morphology and *eps* structure in *P. atlantica*. Photographs of *P. atlantica* colonies are shown on the left (magnification, ×10), while a physical map of the *eps* locus is shown on the right. (A) Mucoid colony. (B) Crenated colony. The right side shows an IS*492* insertion into the *eps* locus. (C) Crenated colony containing two mucoid sectors which resulted from the excision of IS*492*. H, *Hin*dIII; Bg, *Bgl*II.

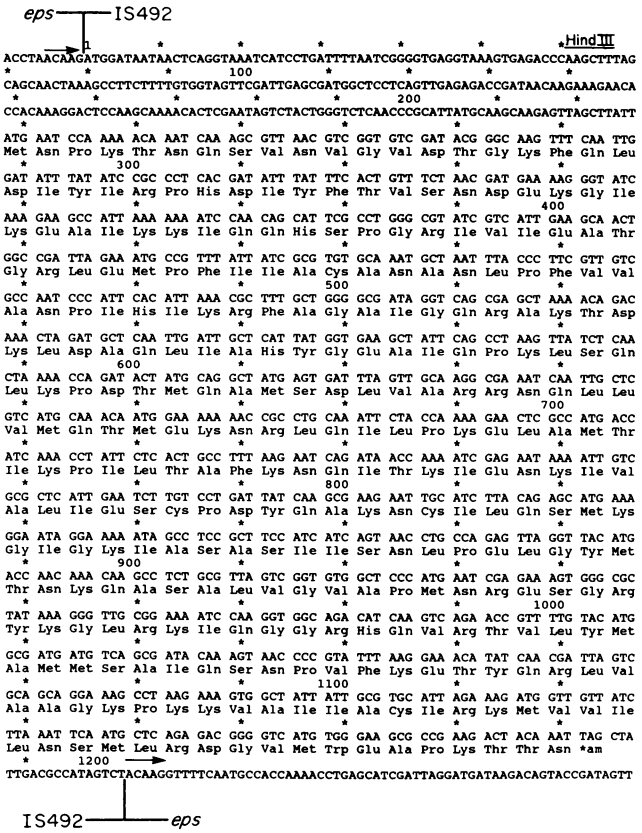

FIG. 2. Nucleotide sequence of IS*492*. The duplicated target site sequences 5′-ACAAG are marked by arrows. *eps*-IS*492* junctions are also delineated. The unique *Hin*dIII restriction site in IS*492* is also shown, as is the open reading frame encoding a protein of 318 amino acids.

of IS*492*; 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 IS*492* 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 IS*492* revealed that IS*492* 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 IS*492*. The termini of IS*492* were defined by sequencing a portion of the *eps* locus with and without the presence of IS*492*. The size of IS*492*, 1,202 nucleotides, is consistent with previous restriction mapping (1.2 kbp). Furthermore, it contains no *Hpa*II sites and has one asymmetric *Hin*dIII site (3). The placement of a variety of other restriction enzyme cleavage sites within the sequence of IS*492* also confirms restriction mapping experiments (data not shown). IS*492* 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 IS*492* is indeed an insertion sequence. Insertion of IS*492* into the *eps* locus generates a 5-bp target duplication, as does insertion of IS*2*, Tn*3*, or bacteriophage Mu (1, 6, 11). Like many other insertion sequence elements, IS*492* 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 IS*492* 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 strong similarity between the IS*492* hypothetical protein and protein sequences present in the National Biomedical Research Foundation data base was found. One unusual characteristic of IS*492* is that it lacks inverted or direct repeats at its termini. This absence of terminal repeats has been observed only in the *Staphylococcus* transposon Tn*554* and in phage Mu (9, 10). IS*492* is the first 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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LITERATURE CITED

1. **Allet, B.** 1979. Mu insertion duplicates a 5 base pair sequence at the host inserted site. Cell **16**:123–129.
2. **Bartlett, D. H., B. B. Frantz, and P. Matsumura.** 1988. Flagellar transcription activators FlbB and FlaI: gene sequences and 5′ consensus sequences of operons under FlbB and FlaI control. J. Bacteriol. **170**:1575–1581.
3. **Bartlett, D. H., M. E. Wright, and M. Silverman.** 1988. Variable expression of extracellular polysaccharide in the marine bacterium *Pseudomonas atlantica* is controlled by genome rearrangement. Proc. Natl. Acad. Sci. USA **85**:3923–3927.
4. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47**:45–148.
5. **Deveraux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
6. **Ghosal, D., H. Sommer, and H. Saedler.** 1979. Nucleotide sequence of the transposable DNA-element IS2. Nucleic Acids Res. **6**:1111–1122.
7. **Hoopes, B. C., and W. R. McClure.** 1987. Strategies in regulation of transcription initiation, p. 1231–1240. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
8. **Iida, S., J. Meyer, and W. Arber.** 1983. Prokaryotic IS elements, p. 159–221. *In* J. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
9. **Kamp, D., and R. Kahmann.** 1980. Two pathways in bacteriophage Mu transposition? Cold Spring Harbor Symp. Quant. Biol. **45**:329–336.
10. **Murphy, E., and S. Lofdahl.** 1984. Transposition of Tn554 does not generate a target duplication. Nature (London) **307**:292–294.
11. **Ohtsubo, H., H. Ohmori, and E. Ohtsubo.** 1979. Nucleotide sequence analysis of Tn3 (Ap): implications for insertion and deletion. Cold Spring Harbor Symp. Quant. Biol. **43**:1269–1277.
12. **Ou, J. T., L. S. Baron, F. A. Rubin, and D. J. Kopecko.** 1988. Specific insertion and deletion of insertion sequence 1-like DNA element causes the reversible expression of the virulence cap-

sular antigen Vi of *Citrobacter freundii* in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85**:4402–4405.
13. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
14. **Silverman, M., and M. Simon.** 1983. Phase variation and related systems, p. 537–557. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
15. **Swanson, J., S. Bergstrom, K. Robbins, O. Barrera, D. Corwin, and J. M. Koomey.** 1985. Gene conversion involving the pilin structural gene correlates with Pilus$^+$ Pilus$^-$ changes in *Neisseria gonorrhoeae*. Cell **47**:267–276.