Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 3923–3927, June 1988
Genetics

Variable expression of extracellular polysaccharide in the marine bacterium *Pseudomonas atlantica* is controlled by genome rearrangement

(recombinant DNA/mobile genetic element)

DOUGLAS H. BARTLETT, MIRIAM E. WRIGHT, AND MICHAEL SILVERMAN

The Agouron Institute, 505 Coast Boulevard South, La Jolla, CA 92037

Communicated by Melvin I. Simon, January 25, 1988 (received for review December 17, 1987)

ABSTRACT **Production of extracellular polysaccharide by the marine bacterium *Pseudomonas atlantica* is a variable trait. Strains that produce extracellular polysaccharide (EPS$^+$) have a mucoid colony phenotype, but during cultivation in the laboratory nonmucoid, EPS$^-$ variants arise that have a crenated colony morphology. This change is reversible since crenated variants rapidly switch to the original mucoid phenotype. We have cloned the locus (*eps*) controlling variable expression of EPS production by screening a recombinant cosmid library for clones that restore EPS production in the crenated variant. By using *eps* as a probe of genomic structure in variant strains, expression of EPS production was found to be controlled by a specific DNA rearrangement. Insertion of a 1.2-kilobase-pair DNA sequence in the *eps* locus results in EPS$^-$, whereas excision of the sequence restores the EPS$^+$ phenotype. Properties of the rearrangement suggest the involvement of a mobile genetic element. The possible significance of this DNA rearrangement to the survival of *P. atlantica* in the ocean is discussed.**

Pseudomonas atlantica attaches to and colonizes a variety of surfaces in the marine environment (1). The ability of a bacterium to elaborate adhesins for binding to a given substratum may be critical to its survival in the ocean because nutrients, which are scarce in seawater, are more abundant on animate surfaces, biofilms, or surfaces with adsorbed organic matter (2). Components associated with the cell envelope such as flagellar and fimbrial appendages, outer membrane proteins, lipopolysaccharides, and extracellular polysaccharides (EPS) function in a large variety of interactions between bacteria and surfaces. With *P. atlantica*, production of an acidic EPS is thought to be a primary determinant of cell adhesiveness (3).

As part of an investigation of the genetic regulation of polysaccharide synthesis in *P. atlantica* we sought to isolate mutants defective in EPS production. However, EPS$^-$ strains were observed to arise spontaneously at frequencies greater than those expected for mutational events. The EPS "variants" are distinguished by their colony morphology. The parental EPS$^+$ phenotype is mucoid (M), and the EPS$^-$ variants display either a crenated (C) or translucent (T) colony morphology. M and nonmucoid (non-M) variants of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* have also been described (4, 5). The genetic mechanisms regulating variable expression of some properties of pathogenic bacteria—i.e., phase variation of flagella in *Salmonella* and pili variation in *Escherichia coli* and *Neisseria*—have been determined (6–8). These variable systems are controlled by complex genomic rearrangements that apparently function to generate antigenic diversity in surface components to allow the pathogens to evade the host immune system. Mechanisms that generate diversity—for example, of adhesive forms—could be important to the survival of marine bacteria as well. Furthermore, insight into novel strategies regulating gene expression might be discovered by exploring the *P. atlantica* system. We report here the isolation of a locus involved in EPS variation and describe a genetic rearrangement controlling its expression.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Bacterial Strains. *P. atlantica*: DB27 (*hsd-1*, Rif^r) and DB50 (*hsd-1*, *recA*::Tn*5#1*, Rif^r) were derived from T6c (refs. 9, 10; unpublished results). Strain Yaphe was a gift from W. Yaphe (11). C forms of these strains were isolated after one to four serial transfers from the air/broth interface from 10 ml of 2216 aerated broth cultures grown in 125-ml Erlenmeyer flasks for 24 hr at 23°C. T and M variants were isolated from papillae that developed from C colonies after incubation on 2216 agar at 30°C for 7 days. *E. coli*: VCS257 [*F*$^-$, *tonA53*, *dapD8*, *lacY1*, *glnV44*, *supE44*, Δ(*gal-uvrB*)*47*, *tyrT58*, *supF58*, *gyrA29*, Δ(*thyA57*), *hsdS3*] was purchased from Stratagene (San Diego, CA). ED8654 (12) is *met*, *gal*, *hsdR*$^-$, *hsdM*$^+$, *supE*, *supF*.

Media. *P. atlantica* strains were cultured at either 23°C or 30°C in 2216 marine medium (Difco) at 75% of the recommended concentration or 28 g/liter. *E. coli* strains were cultured in L broth [10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl per liter] at 37°C. For solid media, 15 g of agar was added per liter of medium. Antibiotics (Sigma) were added to the media at the following concentrations: kanamycin, 50 μg/ml; rifampicin, 100 μg/ml; ampicillin, 100 μg/ml; and tetracycline, 20 μg/ml.

Exopolymer Analyses. Crude EPS was separated from saturated cultures of *P. atlantica* cells grown in 2216 marine medium by sedimenting bacteria at 8000 × *g* for 10 min at 4°C in an RC5-B Sorvall centrifuge. The supernatant was centrifuged a second time and then mixed with an equal volume of isopropyl alcohol for 24 hr at 4°C. The precipitate was collected by centrifugation as above, vacuum dried, and resuspended in 0.7 ml of distilled H_2O. Hexose sugars were analyzed by the anthrone test with glucose as the standard (13). Uronic acids were measured by using the Carbazole analysis of Knutson and Jeanes employing glucuronic acid as the standard (14). Polymer production was calculated as sugar equivalents per ml per OD_{595} of cell culture. Exopolymer preparations were also measured for DNA contamination (A_{260}) to ensure that cell lysis effects were minimal. Lipopolysaccharide production was compared among the variants by the method of Hitchcock and Brown (15).

Abbreviations: EPS, extracellular polysaccharide(s); M, mucoid; C, crenated; T, translucent; IS, insertion sequence(s).

Bacterial Conjugations. Triparental matings were conducted by using strain LE392-4, which contains a kanamycin-sensitive RP4 derivative as the *tra* donor (from K. Timmis, University of Geneva). Matings were performed by using a procedure similar to that described by Ditta *et al.* (16). Approximately 1×10^8 cells from overnight cultures of the cosmid containing *E. coli* strain LE392-4 and the C form of strain DB27 were spotted onto 25-mm (0.45-μm pore) nitrocellulose filters (Microfiltration Systems, Dublin, CA). The filters were incubated at 23°C for 12 hr on 2216 agar before the cells were resuspended and plated onto selective media.

Recombinant Techniques. Genomic libraries of the M and C forms of *P. atlantica* were constructed by using the cosmid vector pMMB33 (17). Cosmid vector "arms" were ligated with donor DNA by the method of Franklin (18). *Sau*3A partially digested donor DNA in the size range of 25–37 kilobase pairs (kbp) was prepared as described by Maniatis *et al.* (19). *In vitro* packaging of ligated DNA was accomplished by using Gigapack Plus (Stratagene) λ DNA packaging extracts according to the manufacturer's instructions. Recombinant clones were transduced into VCS257 on L-kanamycin plates. Transformation into *E. coli*, ligation of DNA, and preparation of plasmid DNA have been described elsewhere (19). Low-copy-number plasmid DNA was prepared by the method of Hansen and Olsen (20). Transposon Tn*5* mutagenesis was performed as described (21). Southern and colony hybridizations were performed as described (19), with the specifications and products outlined by McCarter and Silverman (22).

RESULTS

Variant Colony Phenotypes. Clonal populations of several *P. atlantica* strains (T6c, DB27, and Yaphe) all exhibit three different colonial morphologies. We have characterized these as M, C, and T. M colonies are large, smooth, shiny, and opaque; T colonies are intermediate in size, smooth, and translucent; and C colonies are small, with a greatly wrinkled surface. In liquid culture with 10 mM Ca^{2+}, C cells aggregate near the air/broth interface. This property has facilitated C cell isolation (see *Materials and Methods*). C colonies are the most unstable of the three cell types, particularly when propagated on agar medium at low colony density. After 7 days at 30°C, ≈50% of the cell population will be composed of either the M or T cell type (Fig. 1). However, the M or T form rarely arises from the C form when it is cultured in liquid media or on agar media inoculated at high colony density. Under these conditions, ≈1 M cell arises per 10^5 C bacteria.

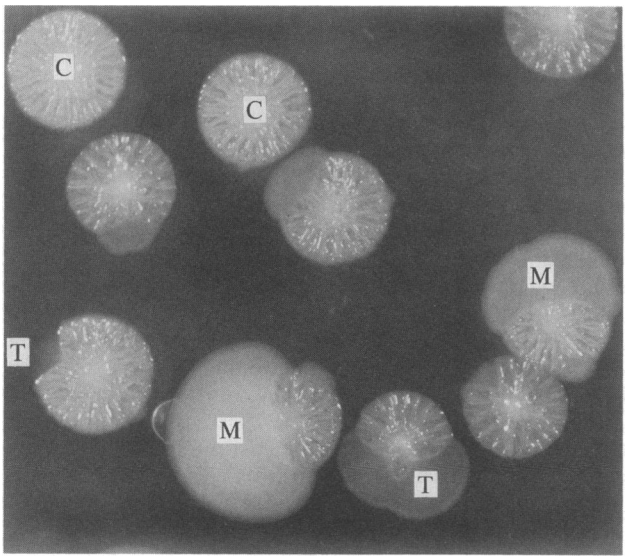

FIG. 1. Colony variation in *P. atlantica*. C colonies switching to the M or T phenotype after 7 days at 30°C on 2216 agar. (×4.)

T and C cells are biochemically distinguishable from M cells by their inability to secrete exopolysaccharide. Uronic acid analysis of EPS from stationary-phase culture supernatants showed that the M variant produced 20 μg of glucuronic acid equivalents per ml per OD_{595}, whereas the C and T cells yielded values of <5% of this amount. The anthrone test for hexose sugars gave similar results. Chemical characterization of the loosely bound polysaccharide from the M parent of DB27 has been performed by others, and glucuronic and galacturonic acids were found to be major constituents of this polymer, in addition to glucose, galactose, and mannose (23). By using this composition of EPS, we calculate that the M form of *P. atlantica* is producing ≈10% of its dry cell weight as EPS. The biochemical basis for the difference between T and C forms is not known. However, no lipopolysaccharide or membrane protein differences among these variants have been detected by silver staining or electrophoretic transfer blot analyses employing whole-cell antisera. We have focused our attention on the M to C variation in strain DB27.

Isolation of *eps* Locus. We hypothesized that variation in EPS production is controlled by a heritable change in the structure of a specific region of *P. atlantica* DNA. The strategy used to isolate this locus was to screen a *P. atlantica* recombinant library for clones that would complement the defect in a C variant (EPS^-) to yield a strain with the M phenotype (EPS^+). Specifically, a chromosomal library of *P. atlantica* DB27 M form (M0) was constructed by using cosmid vector pMMB33, which carries the kanamycin-resistance gene and can be mobilized by conjugation (17). Following *in vitro* packaging and transduction of the bank into the *E. coli* host strain VCS257, ≈2500 independent clones were obtained, representing a >99% probability of representing any given 1-kbp *P. atlantica* chromosomal sequence (24). Pools of cosmids from this library were transferred by means of conjugation into a C variant of *P. atlantica* strain DB27. Strain DB27 is rifampicin resistant, for counterselection of the *E. coli* donor, and restriction defective, to increase the frequency of recovery of exconjugants. After 3 days at 30°C, ≈0.1% of the exconjugants had the M phenotype. Cosmid DNA was isolated from M colonies, transformed into *E. coli* ED8654, and then reintroduced by conjugation back into the C form of *P. atlantica* DB27. Twenty percent of the recombinant molecules (4/20) initially recovered from the M exconjugants were found, when reintroduced into *P. atlantica*, to complement C strain DB27 to the M phenotype (as judged by colony morphology and chemical analysis of EPS). Thus, although variation to the M colony type was responsible for most of the changes observed in the C recipient, it was possible to detect complementation to the M colony form by the introduction of recombinant cosmids. By using restriction analysis, we determined that all EPS^+ cosmids possessed overlapping sets of insert DNAs.

One cosmid, designated pDB200 was saved for further study. Cosmid pDB200 restored EPS production in 10 of 10 independently derived C derivatives of strain DB27. The cloned locus complementing the defect in C *P. atlantica* was designated *eps*, the gene(s) for EPS. A physical map spanning the *eps* locus of pDB200 is shown in Fig. 2*A*. Localization of the DNA segment conferring EPS^+ activity was accomplished in part through the characterization of subclones from pDB200. The smallest complementing subclone contains the 2.5-kbp *Hin*dIII to *Bgl* II fragment, as shown in Fig. 2*B*. Plasmid pDB2006, containing this fragment, exhibits complementation activity in $RecA^+$ and $RecA^-$ strains. Further localization of *eps* was obtained by using transposon Tn*5* mutagenesis. Tn*5* insertions that delineate the *eps* locus are

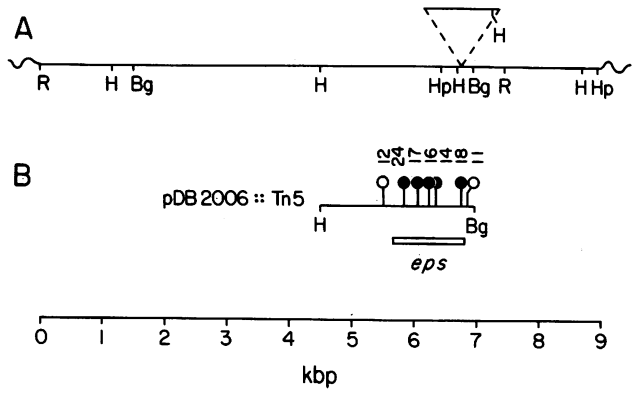

FIG. 2. Localization of *eps* locus. (*A*) Restriction map of the *eps* locus contained on pDB200. R, *Eco*RI; H, *Hin*dIII; Bg, *Bgl* II; Hp, *Hpa* II. Additional *Hpa* II sites outside the *eps* locus are not shown. The position of the 1.2-kbp translocating DNA element present in C cells is displayed above the restriction map. (*B*) Subclone pDB2006 with EPS$^+$ complementation activity. Symbols above the map of pDB2006 represent the location of Tn*5* insertions. Solid symbols represent Tn*5* insertions that prevent EPS$^+$ complementation activity; open symbols mark transposons that do not affect complementation activity. The bar under pDB2006 shows the location of the *eps* locus based upon Tn*5* mutagenesis.

shown in Fig. 2*B*. These insertions define an *eps* locus <1.3 kbp but >0.9 kbp in length.

DNA Rearrangements. To test if EPS switching is associated with a rearrangement of DNA structure in the *eps* locus, chromosomal DNA from one M form (M0) and 10 independently derived C variants (C1–C10) were restricted with *Hpa* II and subjected to Southern blot analysis (25). Plasmid pDB2003, an *eps* subclone that contains the 5.4-kbp *Bgl* II fragment from pDB200, served as the hybridization probe. As seen in Fig. 3*A*, the 2.5-kbp *Hpa* II fragment present in the parental M variant is replaced by a 3.7-kbp *Hpa* II fragment in 9 of 10 C variants derived from it. Similar Southern blot analysis of a cell lineage proceeding M0 → $C1_{(M0)}$ → $M1_{(C1)}$... → $C3_{(M2)}$ revealed the same pattern of change. Designations in subscript denote the particular parental variant from which the variant being described arose. Alteration of DNA structure was not observed in variants $C9_{(M0)}$ and $C3_{(M2)}$. The defect in these rare C variants mapped to the *eps* locus because complementation with *eps*$^+$ cosmid pDB200 was obtained. However, unlike other C variants, these did not switch to the M phenotype. Switching in the opposite direction, of C to the M phenotype, was explored in more detail. Chromosomal DNA from 10 M variants independently derived from variant $C1_{(M0)}$ was likewise restricted with *Hpa* II and probed with pDB2003 (data not shown). In all cases, the 3.7-kbp *Hpa* II fragment present in the $C1_{(M0)}$ genome returned to 2.5 kbp in size in the M variants. From this evidence it is apparent that, in most instances, addition of DNA to the *eps* locus is associated with M to C switching and that this transition is reversible—i.e., all examples of variation from C to M involve the loss of the same amount of DNA. The location of insertions was mapped with greater accuracy by using *Hin*dIII digests because *Hin*dIII cleaves at sites close to and within the rearranged region. Specifically, chromosomal DNA from the same M and C variants examined in Fig. 3*A* was restricted with *Hin*dIII and probed with plasmid pDB4402, which contains *eps* DNA from a C variant (see next section). Given the precision of restriction mapping (±50 base pairs), the results of this analysis suggest that the DNA changes governing EPS variation occur at a single site in the *eps* locus. Furthermore, C → M and M → C transitions occur in RecA$^+$ and RecA$^-$ strains, and the DNA rearrangements associated with these phenotypic changes are identical in RecA$^+$ and RecA$^-$ *P. atlantica* (data not shown).

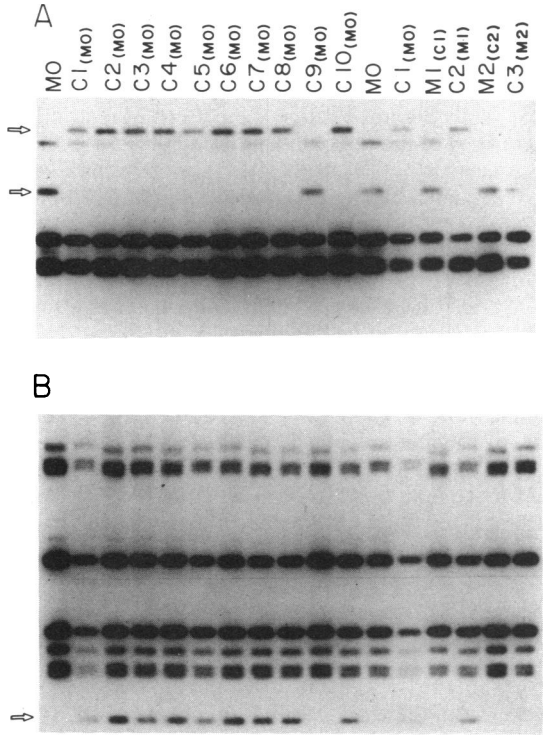

FIG. 3. Genome rearrangement associated with EPS production. (*A*) Chromosomal DNA was prepared from variants M0 and $C1_{(M0)}$ through $C10_{(M0)}$ as well as from a cell lineage proceeding M0 → $C1_{(M0)}$ → $M1_{(C1)}$ → $C2_{(M1)}$ → $M2_{(C2)}$ → $C3_{(M2)}$. DNA restricted with *Hpa* II was probed with pDB2003, which contains the 5.4-kbp *eps Bgl* II fragment. Arrows along the left hand margin point to the hybridizing band differences between DNA from M and C cells. (*B*) Chromosomal DNA from the same variants described in *A*, restricted with *Hin*dIII and probed with pDB4402, which contains the 1.2-kbp translocated DNA element along with the 5.4-kbp *eps Bgl* II fragment. The arrow along the left hand margin points to the hybridizing *Hin*dIII fragment unique to DNA from C cells.

Insertion and Excision of DNA. To more precisely define the rearrangement at the *eps* locus, this region was cloned from a C variant. A genomic library in cosmid vector pMMB33 was prepared from DB27 $C1_{(M0)}$. DNA recombinants containing the *eps* region were detected by probing with the 7.4-kbp *Eco*RI fragment containing *eps* from pDB200 according to the colony-blotting method of Grunstein and Hogness (26). Cosmids from positively hybridizing colonies in the library contained subsets of the DNA fragments previously shown to comprise the *eps* region in M *P. atlantica*. In addition, some cosmids, such as pDB440, contained the 3.7-kbp *Hpa* II fragment unique to the *eps* locus of C variants. As expected, cosmid pDB440 did not complement C cells to the EPS$^+$ phenotype. However, C variants containing pDB440 did switch to EPS$^+$, and 15/16 of the pDB440 cosmids recovered from such M variants were capable of restoring EPS production to C cells. Examination of these *eps*$^+$ cosmids revealed that the 3.7-kbp *Hpa* II fragment was missing and a 2.5-kbp *Hpa* II fragment was present in its place. Either *eps* rearrangement had occurred directly on the cosmid or rearrangement had occurred at the chromosomal *eps* locus, which subsequently moved to the cosmid by recombinational exchange. From comparison of the restriction fragment maps of pDB440 and one of its isogenic *eps*$^+$ derivatives, pDB4401, it was evident that the differences between these cosmids mirrored those that had occurred in the genome of C and M *P. atlantica*. M to C variation is the result of the addition of 1.2 kbp of DNA and C to M variation is the result of loss of 1.2 kbp of DNA from the site, as shown in Fig. 2.

Addition of DNA at *eps* could result from duplication of a sequence in this locus or from insertion of sequence from another region in the genome. The latter was shown to be the case because (*i*) the 1.2 kbp of DNA added at the *eps* locus in EPS$^-$ cells did not contain the pattern of restriction sites characteristic of the region neighboring the *eps* locus and (*ii*) a subclone from pDB440 (pDB4411) consisting almost entirely of sequence from the added 1.2 kbp of DNA present in the *eps*$^-$ locus did not hybridize to sequences in the *eps*$^+$ locus (data not shown).

Multicopy Element. The 1.2-kbp sequence that was shown to be inserted and excised from the *eps* locus is also present in other locations in the genome of *P. atlantica*. Numerous bands observed in Fig. 3*B* are the result of hybridization of the probe to different copies of the 1.2-kbp sequence. By using subclone pDB4411 as a hybridization probe, eight additional fragments were detected in Southern blots of M and C variants (Fig. 4). So, the 1.2-kbp element is obviously present more than once in *P. atlantica*. However, since the arrangement of the additional hybridizing sequences is not known—i.e., unlinked or in tandem arrangement—we cannot compute the precise number of copies. It could be as low as four or higher than 8 in the M variant. Furthermore, with insertion of the 1.2 kbp of DNA in *eps* no hybridization to other fragments is lost, so insertion appears to be a duplicative event. In the C → M transition, the 1.2-kbp DNA element is excised from the *eps* locus, but no change in hybridization at additional locations was observed. So, excision results in a loss of one copy of the DNA element.

DISCUSSION

To investigate the genetic mechanism that controls the variable production of EPS in *P. atlantica*, we have cloned a fragment of DNA that complements the defect in the C (EPS$^-$) variant. Transfer of recombinant plasmids with a locus for EPS production, *eps*, into RecA$^+$ or RecA$^-$ C variants results in expression of a M (EPS$^+$) phenotype indistinguishable from that of the M strain from which the C variants were derived. The size of the region of DNA required for restoration of EPS function, as determined by subcloning and transposon mutagenesis, is between 0.9 and 1.3 kbp, which, assuming an average molecular weight for an amino acid as 110, could encode a single polypeptide of M_r 33,000–48,000. However, further genetic tests must be performed before a definition of a specific gene or genes—i.e., *epsA*, *epsB*, etc.—in this locus can be determined. We do not know what particular function or functions must be provided to complement the defect in the C variants, but *eps* genes could encode proteins required for regulation of gene expression, for activation of sugars, for catalysis of individual steps in polysaccharide polymerization, for translocation of the product out of the cell, or for postpolymerization modification (27). Many *eps* genes are probably required for synthesis and export of polysaccharide, and many of these genes might actually be linked to the locus cloned in this study. However, our test for function is narrow and detects only the locus encoding the function affected by M to C variation.

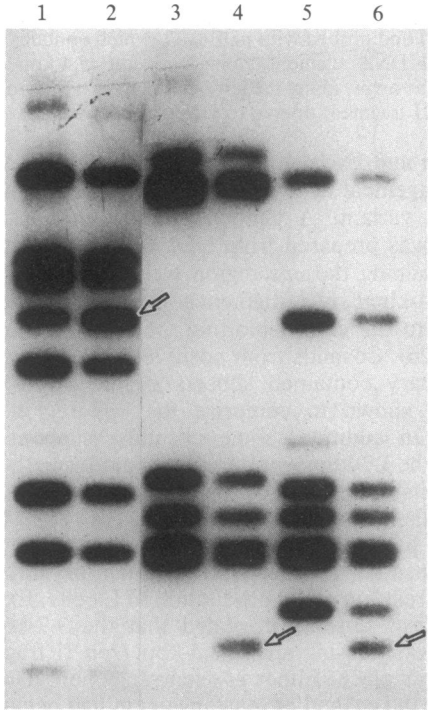

FIG. 4. Multicopy insertion element. Chromosomal DNA from variants M0 and $C1_{(M0)}$ were restricted with various restriction endonucleases and subjected to Southern blot analysis. The probe used was pDB4411, which contains most of the translocated DNA element cloned as a 1.1-kbp *Hin*dIII fragment from pDB440. Lanes 1 and 2, *Hpa* II digests; lanes 3 and 4, *Hin*dIII digests; lanes 5 and 6, *Hin*dIII and *Eco*RI digests. M0 DNA, lanes 1, 3, and 5. $C1_{(M0)}$ DNA, lanes 2, 4, and 6. Arrows point to the position of the extra hybridizing DNA fragment present in DNA from C cells.

Variation between the M (EPS$^+$) form and the C (EPS$^-$) form is caused by a DNA rearrangement. By using the cloned *eps* locus as a probe of the genome structure of EPS variants, insertion of 1.2 kbp of DNA correlated with switching to the C (EPS$^-$) form. Given the limited resolution of restriction mapping, the insertion event appears to be at a single site in the *eps* locus, and excision appears to precisely remove the inserted DNA. The element that inserts and excises from the *eps* locus has properties of an insertion sequence (IS). This element is similar in size to IS and also resides in multiple copies in the genome. Furthermore, movement of the element, like transposition of an IS, is *recA* independent. Insertion of the 1.2-kbp element in *eps* appears to be a duplicative event since the result of insertion is a net increase in the number of copies of the element in the genome of the C variant. However, this result does not necessarily indicate that the insertion of the 1.2-kbp element occurs by a replicative or duplicative mechanism, because interchromosomal transposition by a conservative, nonreplicative mechanism, such as that proposed for most IS (28), would also generate a net increase in the number of copies of the element in the genome.

Several IS from *E. coli* have been characterized with particular regard to their insertion into the *gal* operon. IS*2* insertion occurs at only two sites in the operator domain (29), and IS*4* has been repeatedly observed to integrate into a single site in the *galT* gene (30). Thus, the apparent specificity of insertion in *eps* is consistent with integration properties of IS. Although the element present within the cloned *eps* locus from *P. atlantica* appears to share properties with known IS, no homology between this element and sequences present in either *E. coli*, *P. aeruginosa*, or *P. fluorescens* has been found (data not shown). *P. atlantica*, *P. aeruginosa*, and *P. fluorescens* reversibly switch between M and non-M forms (4, 5). *P. aeruginosa* switching is known to be *recA* independent (31), and *P. fluorescens* non-M forms possess a rough colony morphology similar to the C form of *P. atlantica* (5). These three pseudomonads could share a common mechanism for the variable production of polysaccharide.

Bacterial EPS may function as a protective barrier between a cell and its environment, to concentrate nutrients through electrostatic interactions, or to facilitate attachment and colonization of surfaces (32). The EPS produced by *P. atlantica* has been found to enhance the ability of the organism to attach to surfaces in the marine environment (3). The function of variation in polysaccharide production may

be to create diversity in a population with the result that a small fraction of the bacteria is preadapted to environmental change. The capability of microorganisms to alter their characteristics *in response* to environmental change is surely limited. For example, bacteria can adapt their metabolism to the presence of lactose or phosphate, but can they sense and respond to all of the complex changes that occur in the environment? Also, can bacteria respond rapidly enough to survive the occurrence of hostile situations or to take advantage of favorable circumstances, such as the presence of a nutrient-enriched surface suitable for attachment? An effective, genetically economical, survival strategy could be to employ recombinational switches to generate a diversity of form and function appropriate to certain circumstances before they actually occur.

We are grateful to Dr. Nachum Kaplan for critical review of this manuscript. This research was supported by contracts from the Office of Naval Research (ONR N00014-83-K-0079 and ONR 00014-87-K-0012).

1. Corpe, W. A. (1970) *Dev. Ind. Microbiol.* **11,** 402–412.
2. Norkans, B. (1980) *Adv. Microb. Ecol.* **4,** 51–85.
3. Corpe, W. A., Matsuuchi, L. & Armbruster, B. (1975) in *Proc. Int. Biodegrad. Symp., 3rd,* eds. Sharpley, J. M. & Kaplan, A. M. (Appl. Sci., London), pp. 433–442.
4. Govan, J. R. W. (1975) *J. Med. Microbiol.* **8,** 513–522.
5. Vesper, S. J. (1987) *Appl. Environ. Microbiol.* **53,** 1397–1405.
6. Silverman, M. & Simon, M. (1983) in *Mobile Genetic Elements,* ed. Shapiro, J. A. (Academic, New York), pp. 537–557.
7. Abraham, J. M., Freitag, C. S., Clements, J. R. & Eisenstein, B. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5724–5727.
8. Swanson, J., Bergstrom, S., Robbins, K., Barrera, O., Corwin, D. & Koomey, J. M. (1985) *Cell* **47,** 267–276.
9. Corpe, W. A. (1973) in *Proc. Int. Congr. Mar. Corros. Fouling, 3rd* (Northwestern Univ. Press, Evanston, IL), pp. 598–609.
10. Belas, R., Bartlett, D. & Silverman, M. (1988) *Appl. Environ. Microbiol.* **54,** 30–37.
11. Yaphe, W. (1957) *Can. J. Microbiol.* **3,** 987–993.
12. Murray, N. E., Brammer, W. J. & Murray, K. (1977) *Mol. Gen. Genet.* **150,** 53–61.
13. Dische, Z. (1962) *Methods Carbohydr. Chem.* **1,** 490–491.
14. Knutson, C. A. & Jeanes, A. (1968) *Anal. Biochem.* **24,** 470–481.
15. Hitchcock, P. C. & Brown, T. M. (1983) *J. Bacteriol.* **154,** 269–277.
16. Ditta, G., Stanfield, S., Corbin, D. & Helinski, D. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7347–7351.
17. Frey, J., Bagdasarian, M., Feiss, D., Franklin, F. C. H. & Deshusses, J. (1983) *Gene* **24,** 299–308.
18. Franklin, F. C. H. (1985) in *DNA Cloning: A Practical Approach,* ed. Glover, D. M. (IRL, Arlington, VA), Vol. 1, pp. 165–184.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Hansen, J. B. & Olsen, R. H. (1978) *J. Bacteriol.* **135,** 227–238.
21. Boyd, A., Krikos, A. & Simon, M. (1981) *Cell* **26,** 333–343.
22. McCarter, L. L. & Silverman, M. (1987) *J. Bacteriol.* **169,** 3441–3449.
23. Uhlinger, D. J. & White, D. C. (1983) *Appl. Environ. Microbiol.* **45,** 64–70.
24. Clarke, L. & Carbon, J. (1976) *Cell* **9,** 91–99.
25. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
26. Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
27. Sutherland, I. W. (1979) in *Microbial Polysaccharides and Polysaccharases,* eds. Berkeley, R. C. W., Gooday, G. W. & Ellwood, D. C. (Academic, London), pp. 1–34.
28. Grindley, N. D. F. & Reed, R. R. (1985) *Annu. Rev. Biochem.* **54,** 863–896.
29. Ahmed, A., Birdwell, K. & Musso, R. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 141–151.
30. Klaer, R., Pfeifer, D. & Starlinger, P. (1980) *Mol. Gen. Genet.* **178,** 281–284.
31. Ohman, D. E., West, M. A., Flynn, J. L. & Goldberg, J. B. (1985) *J. Bacteriol.* **162,** 1068–1074.
32. Costerton, J. W., Irvin, R. T. & Cheng, K.-J. (1981) *Annu. Rev. Microbiol.* **35,** 299–324.