A New Azotobacter vinelandii Mannuronan C-5-Epimerase Gene (algG) Is Part of an alg Gene Cluster Physically Organized in a Manner Similar to That in Pseudomonas aeruginosa

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Alginate is an unbranched polysaccharide composed of the two sugar residues β-1-mannuronic acid (M) and α-1-guluronic acid (G). The M/G ratio and sequence distribution in alginites vary and are of both biological and commercial significance. We have previously shown that a family of highly related mannuronan C-5-epimerase genes (algE1 to -E5) controls these parameters in Azotobacter vinelandii, by catalyzing the Ca<sup>2+</sup>-dependent conversion of M to G at the polymer level. In this report, we describe the cloning and expression of a new A. vinelandii epimerase gene (here designated algG), localized 29 nucleotides downstream of the previously described gene algI. Sequence alignments show that algG does not belong to the same class of genes as algE1 to -E5 but that it shares 66% sequence identity with a previously described mannuronan C-5-epimerase gene (also designated algG) from Pseudomonas aeruginosa. A. vinelandii algG was expressed in Escherichia coli, and the enzyme was found to catalyze epimerization in the absence of Ca<sup>2+</sup>, although the presence of the cation stimulated the activity moderately. Surprisingly, all activity was blocked by Zn<sup>2+</sup>. P. aeruginosa AlgG has been reported to contain an N-terminal export signal sequence which is cleaved off during expression in E. coli. This does not happen with A. vinelandii AlgG, which appears to be produced at least partly in an insoluble form when expressed at high levels in E. coli. DNA sequencing analyses of the regions flanking algG suggest that the gene is localized in a cluster of genes putatively involved in alginate biosynthesis, and the organization of this cluster appears to be the same as previously described for P. aeruginosa.

Alginate is a polysaccharide produced by brown seaweeds and certain bacteria. In the seaweeds, the polymer probably serves an important function in determining the mechanical properties of the algal tissues. The combination of the availability of large quantities of seaweeds and the commercially interesting physical properties of alginites forms the basis for numerous applications of this polymer in industry and advanced biotechnology (25). The majority of these applications are based on the viscosity properties of alginate and on the ability of the polymer to form gels in the presence of divalent cations like Ca<sup>2+</sup>.

Bacterial alginate synthesis has been identified and studied in organisms belonging to the genera Pseudomonas and Azotobacter (4, 10, 11, 15). Most of our knowledge on the genetics of alginate synthesis originates from studies of Pseudomonas aeruginosa, and these studies are mainly motivated by the problems that this pathogen causes for patients suffering from the disease cystic fibrosis. These problems strongly correlate with alginate production through the serious effects the polymer has on the respiratory system of the patients (16). In both Azoto-
bacter vinelandii and P. aeruginosa, alginate is synthesized as an extracellular polysaccharide in vegetatively growing cells, but in A. vinelandii, the polysaccharide also plays a crucial role in the unique ability of this organism to enter a so-called cyst stage. In this state, the cells are protected from certain environmental stress conditions, like dehydration (20). A. vinelandii cysts are coated with an outer protective layer containing alginites of varying composition. This structural variation, which is also observed in different parts of individual seaweed plants, involves differences in the fractional ratios and distributions of the two sugar monomers β-1-mannuronic acid (M) and α-1-guluronic acid (G) along the unbranched polymer chains (13, 20). Bacterial alginites are in addition acetylated, while this is not the case for alginites from seaweeds (23).

It is well established that alginites containing blocks of G residues can form strong gels in the presence of divalent cations like Ca<sup>2+</sup> (25). If the G residues are distributed in a more alternating sequence pattern, most of the gel-forming properties are lost (26). The sequence distribution of M and G residues is therefore important from both a biological and an application point of view. Alginites produced by Pseudomonas species do not contain G blocks, while such structures are found in brown seaweed and A. vinelandii alginites (13, 23). In two recent papers, we showed that the sequence distribution of M and G residues in A. vinelandii alginites is determined by the gene products (mannuronan C-5 epimerases) of a family of highly related genes (6, 7). The epimerases have the unusual property of converting M to G at the polymer level, and this reaction can take place in vitro in the absence of any cofactors other than the divalent cation Ca<sup>2+</sup>. The existence of a mannuronan C-5 epimerase gene (algG) in P. aeruginosa has also been reported previously (9). Alignment analyses at the DNA and protein level showed that the P. aeruginosa gene (and its product) was quite different from its counterparts (algE1 to -E5) in A. vinelandii (7).

The P. aeruginosa algG gene is localized within a cluster of genes encoding enzymes directly involved in different stages of the alginate biosynthetic pathway (16). Such genes were not found to be associated with the A. vinelandii epimerase genes. It has, on the other hand, been shown that probes prepared from P. aeruginosa alg genes hybridize against A. vinelandii genomic DNA (8). These data therefore indicated that there exists at least some level of similarity in the genes controlling

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alginate biosynthesis in the two organisms. Recent experiments have verified this hypothesis directly by the identification of an A. vinelandii gene (algG) encoding an outer membrane protein thought to be involved in alginate export (18). A similar gene (algE), encoding an outer membrane protein, has previously been reported in P. aeruginosa (2, 19). Furthermore, an A. vinelandii gene (designated algD) analogous to P. aeruginosa algD (encoding GDP-mannose dehydrogenase) was recently reported and was shown to be required for alginate synthesis in vivo in A. vinelandii (1). In P. aeruginosa, algD is localized next to algE, and we show here that a gene similar to algG is found in the same relative position in A. vinelandii. On the basis of these data and further DNA sequencing analyses, we conclude that the alginate biosynthetic genes may be similarly organized in the two organisms but that A. vinelandii (presumably in contrast to P. aeruginosa) has two systems for epimerization of M to G.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** Strains and plasmids are listed in Table 1. Escherichia coli cells were grown at 37°C in LB broth or on L agar (21) supplemented with ampicillin (100 μg/ml) when relevant. P. aeruginosa was grown in liquid medium at 32°C, as previously described by Skjåk-Bræk et al. (22), or at 18°C on 3% agar medium containing (per liter) 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 1 g of glucose.

**DNA manipulations and cloning of algD.** DNA was sequenced according to the method of Sanger et al. (23). Plasmids used for determination of sequences homologous to the P. aeruginosa alg genes were pHE106 (algD homolog), pHE107 (algF homolog, C1 [see Fig. 5]), pHE105 (algG homolog, C2 [see Fig. 5]), pHE106 (alg44 homolog), and pHE104-pHE110 (algD homolog). Vector primers were used in all these DNA sequencing procedures, while algG was sequenced by primer walking in both directions. The sequence homologous to algG was obtained from the data shown in Fig. 1. Standard recombinant DNA procedures were performed according to protocols described in the work of Sambrook et al. (21), except for transformations, in which the protocol of Chung et al. (3) was also used. Labeling of DNA for the Southern hybridization analysis was performed by the digoxigenin system from Boehringer Mannheim. The DNA fragment containing algG originated from the same recombinant EMBL3 plasmid (BR100) as algG (18), and algG was identified by sequencing of the region downstream of algG with plasmid pBHRS9 as template. High-level expression of algG was obtained by subcloning into the expression vector pQE60. The cloned fragment was prepared by PCR amplification with the synthetic oligonucleotides 5'-CCACGCGGACGCTGAGACGATGACCAGTGAAG3' (5' end of algG) and 5'-CATTTGCTGTTGTAGAGATCTTCATGTT3' (3' end of algD) and 5'-CATTTGCTGTTGTAGAGATCTTCATGTT3' (3' end of algG). In this strategy, a NeoI site was introduced near the 5' end and a BglII site was introduced near the 3' end of the fragment. Both sites were used for cloning of the PCR fragment into the NeoI-BamHI sites of the vector pQE60. The use of the NeoI site made it possible to position the first ATG optimally relative to the ribosome-binding site in the vector.

**Preparation of [3H]alginate.** P. aeruginosa was first grown for 3 days in liquid medium, and 0.5 ml of the culture was then plated on each of six agar plates (diameter, 14.5 cm) and incubated for 24 h at 37°C on a shaker. The plates were washed once with 10 ml of 0.9% NaCl and then cut out and suspended in a total volume of 0.9 ml of 0.9% NaCl. A sample of strain JM109(pBHR60) were grown overnight in the presence of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and washed once in 10 mM HEPES (N-(N,N-dimethylaminopropyl)-N-ethanesulfonic acid) pH 7.4. Whole-cell lysates were prepared by resuspending the cells (10 times concentrated) in gel loading buffer. The cytosol fraction was prepared by disrupting the cells in a French press and by sedimenting cell debris with centrifugation (40,000 × g) for 1 h. The pellet (insoluble fraction) was resuspended in the above HEPES buffer. AlgG was identified as a protein band at 55 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in strains induced to express algG and not in vector (pQE60) control strains.

For measurements of AlgG activity, JM109(pBHR60) cells were inoculated at 1% from overnight cultures, and after 4.75 h, IPTG was added at 0.5 mM. Cells (900 ml) were harvested 2.5 h after IPTG induction, resuspended in 60 ml of 50 mM Tris-2-m M dithiothreitol-1 mM phenylmethylsulfonyl fluoride, and sonicated. After centrifugation at 30,000 × g for 30 min, the pellet was resuspended in 6 ml of 50 mM Tris-3% CHAPS [3-[3-cholamidopropyl]dimethyl-ammonio]-1-propanesulfonate]-0.5 mM NaCl (pH 7.0) and stored overnight without significant loss of activity. It was then centrifuged again at 30,000 × g for 30 min, and the supernatant was subjected to filtration sterilization. No activity was lost

**TABLE 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Characteristics</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli JM109</td>
<td>recA1 supF44 endA1 hsdR17 gyrA96 relA1 thi-1 (rac-proAB)</td>
<td>27</td>
</tr>
<tr>
<td>P. aeruginosa 8830</td>
<td>his-1 AlgI; stable mucoid mutant</td>
<td>5</td>
</tr>
<tr>
<td>Phage BR100</td>
<td>EMBL3A derivative containing a 17-kb insert from A. vinelandii; encodes algG</td>
<td>18</td>
</tr>
</tbody>
</table>

**Plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pQE60</td>
<td>Ap’, ColE1 replicon</td>
</tr>
<tr>
<td>pUC19</td>
<td>Ap’, ColE1 replicon</td>
</tr>
<tr>
<td>pUC128</td>
<td>Ap’, ColE1 replicon</td>
</tr>
<tr>
<td>pBHRS9</td>
<td>Derivative of pUC19 in which a 2.6-kb EcoRI fragment containing A. vinelandii algG was inserted</td>
</tr>
<tr>
<td>pBHRS0</td>
<td>Derivative of pQE60 in which a 1.7-kb PCR-amplified NeoI-BglII fragment containing algG was inserted</td>
</tr>
<tr>
<td>pHE102</td>
<td>Derivative of pUC128 in which a 2.8-kb XhoI-Smal DNA fragment from BR100 was inserted. Contains the 3’ end of the A. vinelandii algG gene and the two boxes having homology with algX and algL from P. aeruginosa.</td>
</tr>
<tr>
<td>pHE104</td>
<td>Derivative of pHE102 in which a 1.1-kb EcoRV-XhoI (poly linker at Smal end) DNA fragment was deleted</td>
</tr>
<tr>
<td>pHE105</td>
<td>Derivative of pUC19 in which a 3.3-kb EcoRI-Smal DNA fragment from BR100 was inserted. Contains sequences homologous to the P. aeruginosa alg8 and alg44 genes</td>
</tr>
<tr>
<td>pHE106</td>
<td>Derivative of pHE105 in which a 1.0-kb EcoRI-NcoI DNA fragment was deleted</td>
</tr>
<tr>
<td>pHE107</td>
<td>Derivative of pUC19 in which a 6.0-kb Smal-EcoRI DNA fragment from BR100 was inserted. Contains sequences homologous to algD and alg8</td>
</tr>
<tr>
<td>pHE108</td>
<td>Derivative of pHE107 in which a 5-kb region was deleted. The region spans the HindIII site in the vector poly linker (Smal end of insert) to the XhoI site near box B in Fig. 5</td>
</tr>
<tr>
<td>pHE110</td>
<td>Derivative of pHE102 in which a 1.7-kb region from the XhoI to the EcoRV site was deleted. Contains sequences homologous to algL, in Fig. 5</td>
</tr>
</tbody>
</table>

**Visualization of AlgG by SDS-PAGE and measurements of AlgG activity.** Cells of strain JM109(pBHR60) were grown overnight in the presence of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and washed once in 10 mM HEPES (N-(N,N-dimethylaminopropyl)-N-ethanesulfonic acid) pH 7.4. Whole-cell lysates were prepared by resuspending the cells (10 times concentrated) in gel loading buffer. The cytosol fraction was prepared by disrupting the cells in a French press and by sedimenting cell debris with centrifugation (40,000 × g) for 1 h. The pellet (insoluble fraction) was resuspended in the above HEPES buffer. AlgG was identified as a protein band at 55 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in strains induced to express algG and not in vector (pQE60) control strains.
in this last centrifugation and filtration step. The filtered solution was used for activity measurements. Activity assays (see below) demonstrated that the AlgG activity in the cytosol fraction obtained directly after centrifugation was higher than that in the corresponding pellet, calculated as disintegrations per minute per original cell mass. However, by using the pellet for activity assays we obtained higher activities per weight unit of total protein. This turned out to be important, since the measured activities were low (see Table 2). 

[3H]alginate (84,000 dpm/mg) was used as substrate in the activity assays (24). The incubation mixtures (0.6-ml total volume) contained 0.25 mg of [3H]alginate per ml, 0.1 ml of cell extract, and 30 mM Tris (pH 7.0), and divalent cations were added as indicated. The mixtures were incubated at 37°C for 12 h, and epimerase activities were monitored as released [3H], as previously described (6) except that the alginate was precipitated with 0.8 ml of isopropanol.

N-terminal sequencing of AlgG. Aliquots of sonicated IPTG-induced cells from JM109(pBHR60) were loaded directly on an SDS-PAGE gel and subjected to amino-terminalsequencing, as previously described (6).

Computer analyses. The program PC/GENE (version 6.7) was used to determine the predicted isoelectric point of A. vinelandii AlgG, while alignments were performed by the use of Seqaid.

Nucleotide sequence accession number. The algG nucleotide sequence data were deposited in the Genbank database under the accession number X87973.

RESULTS

Cloning and sequencing of a new mannuronan C-5-epimerase gene. The region downstream of the previously described algJ gene was subjected to DNA sequencing, and one long open reading frame (ORF) was identified (Fig. 1). A 1.5-kb DNA fragment containing this ORF was identified (Fig. 1). A 1.5-kb DNA fragment containing this ORF was subcloned by PCR from the same recombinant A. subcloning algJ contained the corresponding site of the ATG expression vector pQE60. The resulting plasmid, pBH60, was used to express the polypeptide encoded by algG. Figure 3, lanes 1 and 2, shows that in the presence of IPTG induction of a protein of 58 kDa was efficiently expressed in cells containing pBH60 but is absent in cells containing the vector only. The protein was also visualized in the cytosol fraction (lanes 3 and 4) and in the pellet (insoluble fraction) obtained by centrifugation of the cells disrupted in a French press (lanes 5 and 6). This is in agreement with the results obtained in the activity assays (see below).

P. aeruginosa AlgG has been reported to contain a N-terminal export signal sequence which is cleaved off (35 amino acids) when the protein is expressed in E. coli (9). We have therefore also sequenced the N terminus of A. vinelandii AlgG expressed in E. coli and found that the sequence is X (uniden-
tified)-V-Q-R-K. This sequence corresponds to the start of the deduced ORF shown in Fig. 1 and 2, and the data thus indicate that no signal peptide is cleaved off during production in *E. coli*.

**Measurement of epimerase activity.** Epimerase activity was assayed in extracts prepared from uninduced and IPTG-induced cells containing pBHR60. Under uninduced conditions, the activities were found to be low but detectable in this sensitive assay (not shown). The highest activity (100%) was observed in extracts from induced cells and with an assay buffer containing 5 mM CaCl₂ and 10 mM MgCl₂ (Table 2). In the absence of any of these metals, 39% of the activity was still retained. This remaining activity was also found to be essentially insensitive to the addition of 10 mM Na₂-EDTA, indicating that there is no absolute requirement for divalent metal ions. The properties of AlgG are therefore different from those of the strictly Ca²⁺-dependent *A. vinelandii* epimerases (AlgE1 to -E5) previously described. Surprisingly, the addition of as little as 1 mM ZnCl₂ to the assay mixture completely abolished the activity, while MnCl₂ and Na₂MoO₄ had no significant effect (not shown).

The physical organization of many *alg* genes is similar in *P. aeruginosa* and *A. vinelandii*. On the basis of previously reported data (18) and the experiments reported here, it seems clear that *algJ* and *algG* correspond to and are organized in a manner similar to that of *algE-algG* in *P. aeruginosa*. Because of the previous identification of a complex system of epimerase genes in *A. vinelandii*, it was obviously of interest to know whether *algG* is a member of another multicopy gene family. To analyze this, we hybridized *A. vinelandii* genomic DNA against an *algG* probe, and the results of this analysis strongly indicate that there is only one copy of the gene in the genome (Fig. 4).

Since *algE* and *algG* are part of a cluster of *alg* genes in *P. aeruginosa*, it seemed probable that more *alg* genes might be localized in the *A. vinelandii* DNA sequences flanking *algE-algG*. A restriction endonuclease map of the insert in BR100 was constructed (Fig. 5A), and several of the fragments flanking *algG* were then subjected to partial DNA sequencing. The sequences were translated into putative amino acid sequences in all three reading frames in both orientations and then were aligned with the previously reported *alg* gene sequences from *P. aeruginosa*. In each case, one of the reading frames was translated into an amino acid sequence that was essentially identical to the previously reported sequence.

**TABLE 2. Effects of divalent cations on AlgG activity (percent)**

<table>
<thead>
<tr>
<th>Conc (mM) of salt</th>
<th>CaCl₂</th>
<th>MgCl₂</th>
<th>ZnCl₂</th>
<th>Na₂-EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>64</td>
<td>36</td>
<td>ND</td>
<td>38</td>
</tr>
<tr>
<td>10</td>
<td>59</td>
<td>65</td>
<td>ND</td>
<td>37</td>
</tr>
<tr>
<td>15</td>
<td>59</td>
<td>65</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*a* One hundred percent activity was obtained in the presence of 5 mM CaCl₂-10 mM MgCl₂ and was measured as 2,020 dpm (blank subtracted). The blank value was 99 dpm. In the absence of added salts, the activity was 39%.

*b* ND, not determined.

**FIG. 2.** Comparison of the AlgG sequences from *A. vinelandii* and *P. aeruginosa*. The Pseudomonas sequence was obtained from GenBank (accession number U06720). The export signal sequence in *P. aeruginosa* is underlined (9). **A.v,** *A. vinelandii; P.a,* *P. aeruginosa.*

**FIG. 3.** Visualization of *algG* expression in *E. coli* by SDS-PAGE. Lanes 1, 3, and 5, whole-cell extracts, cytosol, and insoluble fraction (respectively) from JM109(pQE60). Lanes 2, 4, and 6, the same fractions from IPTG-induced JM109(pBHR60). The cytosol and insoluble fractions correspond to about 20% of the cells relative to the whole-cell extracts. The numbers to the left refer to a molecular mass standard (kilodaltons).
frames showed significant homology to specific parts of alg genes from this organism (Fig. 5B). Furthermore, the homologous sequences from A. vinelandii were all in the same orientation as in P. aeruginosa, and the relative positioning was the same. On the basis of these data, we conclude that the A. vinelandii genome encodes putative genes having extensive homology to the P. aeruginosa algD, alg8, alg44, algE, algG, algX, and algL genes, and they are all physically organized in the same way in the two organisms.

DISCUSSION

The structures of P. aeruginosa alginites are much simpler than those found in the complex polymer mixtures produced by A. vinelandii. It therefore is not too surprising to find that these differences are also reflected at the genetic level. The identification of a homolog of the P. aeruginosa algG gene in A. vinelandii was, on the other hand, unexpected by us, since it means that the genome of this organism encompasses two classes of epimerization genes. The biological significance of having two such systems for epimerization is not clear. However, alginate is produced by vegetatively growing A. vinelandii cells, and since the algG gene was found to be localized in a putative alg biosynthesis cluster (as in P. aeruginosa), it seems probable that A. vinelandii algG is expressed under vegetative growth conditions. The original cloning of the first algE gene (algE2) was based on a protein expressed in vegetatively growing cell cultures (5), and it therefore seems likely that both systems for epimerization are used by the cells under standard laboratory growth conditions. It is also clear that there are more sequences having homology with algE1 to -5 in the A. vinelandii genome (reference 7 and unpublished data), and until the complete set of genes involved in epimerization has been characterized, it will be difficult to obtain a full understanding of the role of these enzymes in the biology of A. vinelandii. Such studies will have to be performed under a variety of environmental conditions, since it is probable that some of the epimerase genes play a role in cyst formation under conditions of environmental stress.

The recombinantly produced AlgG protein from A. vinelandii appears to have a very low specific activity. On the basis of SDS-PAGE gels, expression levels are clearly very high, but in spite of this, we were unable to obtain a sufficiently high activity to study the epimerization pattern by nuclear magnetic resonance spectroscopy. We believe that the most probable explanation of this is that the majority of the protein is produced in an inactive or poorly active form. The reason for this is not clear but may possibly be related to the lack of process-

FIG. 4. Southern hybridization of algG against restriction endonuclease-digested chromosomal DNA from A. vinelandii. A 1.1-kb DNA fragment spanning the region from the HindIII site at nucleotide 710 (Fig. 1) to the polylinker HindIII site downstream of algG in pBR322 was used as a probe. Lane 1, KpnI digest. Lane 2, BglII digest. Lane 3, EcoRI digest. The numbers to the left refer to a molecular mass standard (kilobases). No signals were observed at lower molecular masses than those indicated.

FIG. 5. (A) Restriction endonuclease map of the insert in the recombinant EMBL3 phage BR100. Black boxes on this map indicate DNA sequences used for alignments against P. aeruginosa alg genes. Each vertical bar in the map represents 1 kb. S, Smal; X, XhoI; E, EcoRI; N, NcoI; V, EcoRV. There are many NcoI and EcoRV sites in the BR100 insert, and only those used for cloning are shown. The inserts in the plasmids used for the characterizations are indicated below the map. The corresponding map of the previously reported P. aeruginosa genes (16) is shown above the A. vinelandii restriction map in the same scale. Each black box in the P. aeruginosa map has homology with the corresponding box in the A. vinelandii map. (B) Sequence alignments between P. aeruginosa alg genes and A. vinelandii DNA flanking algD-algG. The alignments are based on previously characterized P. aeruginosa alg genes and sequences from parts (B, C1, C2, D, E, and F) of the inserts in the plasmids shown in panel A. Note that the sequence 5′ to the black box (E) in the A. vinelandii algX homolog has also been determined (Fig. 1), but the deduced amino acid sequence did not have much homology with the P. aeruginosa AlgX protein. The same was true for the first nine deduced amino acids following immediately downstream of box D. With these two exceptions, all sequenced regions are shown, and the homology percentages in the aligned regions were calculated to be 64 (algD), 84 (algE), 58 (alg44), 57 (algX), and 69 (algL). The P. aeruginosa sequences were obtained from GenBank and had the following accession numbers: Y00337 (algD), L22611 (algE and alg44), M37181 (algE), L27829 (algX = alg60), and U09724 (algL). The A. vinelandii algD sequence reported by Campos et al. (1) is identical to the one shown here. The alignments of algD and algF have been reported previously (18), and the algG comparisons are shown in Fig. 2. A, XhoI. A. vinelandii; P, P. aeruginosa.
ing in E. coli, in contrast to what was observed with P. aeruginosa AlgG (9).

If the algG gene products behave similarly in the two species, it is likely that these enzymes cannot form G blocks, although this has not been shown directly, even in P. aeruginosa. Another interesting observation is that at least A. vinelandii algG does not seem to require Ca\(^{2+}\) for its activity, while this is an absolute necessity for the algE class of epimerases (6). It is tempting to assume that this difference is somehow taken advantage of by the organism, particularly since this cation is of crucial importance via its effects on alginate gel formation.

The finding that the genes putatively involved in other aspects of alginate biosynthesis are almost identical in physical organization in P. aeruginosa and A. vinelandii demonstrates that the biosynthetic machinery has a common evolutionary origin in the two organisms. It therefore seems likely that the complex epimerization system apparently unique to A. vinelandii is a trait that evolved later primarily to allow formation of the alginate structure in Azotobacter vinelandii. Mol. Microbiol. 7:119–731.


