Alginate formation in *Azotobacter vinelandii* UWD during stationary phase and the turnover of poly- β -hydroxybutyrate

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***Azotobacter vinelandii* UWD is a mutant of strain UW that is defective in the respiratory oxidation of NADH. This mutation causes an overproduction of polyhydroxyalkanoates (PHAs), as polyester synthesis is used as an alternative electron sink. Since PHAs have potential for use as natural, biodegradable plastics, studies of physiology related to their production are of interest. Alginate production by this strain is limited to $< 11 \mu\text{g}$ (mg cell protein) $^{-1}$, which permits high efficiency conversion of carbon source into PHA. However, $\geq 400 \mu\text{g}$ (mg cell protein) $^{-1}$ was formed when UWD cells were oxygen-limited and in the stationary phase of growth. Alginate formation was fuelled by PHA turnover, which was coincident with the synthesis of alkyl resorcinols, under conditions of exogenous glucose limitation. However, alginate production was a phenotypic and reversible change. Alginate production was stopped by interruption of *algD* with *Tn5lacZ*. LacZ activity in UWD was shown to increase in stationary phase, while LacZ activity in a similarly constructed mutant of strain UW did not. Transcription of *algD* in strain UWD started from a previously identified RpoD promoter and not from the AlgU (RpoE) promoter. This is because strain UWD has a natural insertion element in *algU*. Differences between strain UW and UWD may reside in the defective respiratory oxidation of NADH, where the NADH surplus in strain UWD may act as a signal of stationary phase. Indeed, a backcross of UW DNA into UWD generated NADH-oxidase-proficient cells that failed to form alginate in stationary phase. Evidence is also presented to show that the RpoD promoter may be recognized by the stationary phase sigma factor (RpoS), which may mediate alginate production in strain UWD.**

Keywords: *Azotobacter vinelandii*, alginate, *algU*, poly- β -hydroxybutyrate, RpoS

INTRODUCTION

Azotobacter vinelandii strain UWD has been studied intensively as a candidate for the commercial production of polyhydroxyalkanoates (PHAs), which can be used as natural, biodegradable plastics (Chen & Page, 1997; Page, 1992; Page *et al.*, 1992). A mutation in strain UWD limits the respiratory oxidation of NADH, which results in a constitutive PHA hyperproduction phenotype as the synthesis of poly- β -hydroxybutyrate (PHB) is used as an alternative electron sink to consume excess

NAD(P)H during growth (Page & Knosp, 1989; Page & Manchak, 1994). Thus PHA production is increased by aerobic conditions, which promote rapid sugar catabolism and the rapid generation of NAD(P)H (Page, 1990).

The PHB-hyperproduction phenotype was originally created in *A. vinelandii* strain 113 by nitrosoguanidine mutagenesis (Page & Knosp, 1989). However, strain 113 is unsuited to commercial PHB production because it forms copious amounts of extracellular alginate. Thus strain UWD was created by transformation of the PHB-hyperproduction trait of strain 113 into *A. vinelandii* strain UW (Page & Knosp, 1989), an alginate-minus host (Bush & Wilson, 1959). The capsule-minus geno-

Abbreviations: PHA, polyhydroxyalkanoate; PHB, poly- β -hydroxybutyrate.

type of strain UW is extremely stable and spontaneous revertants to the capsule-positive phenotype have never been reported.

However, the alginate biosynthetic pathway is intact in strain UW and very limited alginate production has been observed after prolonged incubation (Page, 1983). The *A. vinelandii* biosynthetic pathway has been shown previously to be identical to that found in *Pseudomonas aeruginosa* and many of the genes are conserved (Fialho *et al.*, 1990; Gacesa, 1998; Rehm *et al.*, 1996). The transcriptional activation of the GDP-mannose dehydrogenase gene (*algD*) is a key point in pathway regulation (Campos *et al.*, 1996) and is mediated by the alternative sigma factor AlgU, a homologue of RpoE (Martinez-Salazar *et al.*, 1996). Recently it was shown that *A. vinelandii* strain UW136 has a natural insertion element in the *algU* gene (Martinez-Salazar *et al.*, 1996), which may explain the alginate-minus phenotype of strains derived from UW.

We have recently been using O₂-limited culture for the production of higher yields of PHA in strain UWD (Chen & Page, 1997). The promotion of PHA formation by O₂ limitation is commonly used with other bacteria (Anderson & Dawes, 1990) and does increase PHA yield in strain UWD. However, we have observed that the culture can become viscous, leading to foam stabilization and difficulty harvesting the cells (unpublished results). In this study, we show that strain UWD can form a significant amount of alginate in an AlgU-independent manner during the turnover of PHB.

METHODS

Bacterial cultures and growth conditions. The strains used in this study included *Azotobacter vinelandii* UW (ATCC 13705), its rifampin-resistant derivative UW136 (Dr P. E. Bishop, North Carolina State University, Raleigh, NC, USA), the PHB-hyperproducing strain UWD (ATCC 53799) (Page & Knosp, 1989) and the capsule-positive strain O (ATCC 12518). *A. vinelandii* strain U5 had *algD* insertionally inactivated by Tn5*lacZ* mutagenesis of strain UW136 (Campos *et al.*, 1996). Strain DU5 (*algD*::Tn5*lacZ* in strain UWD) and strain DC (UWD backcrossed with UW DNA) were constructed in this study by transformation (Page & von Tigerstrom, 1979).

The strains were routinely grown in Burk's buffer salts (Page & Sadoff, 1976), supplemented with ferric citrate to bring the total iron concentration to 50 µM, 2% (w/v) glucose and 15 mM ammonium acetate (Burk's medium). The medium for optimal PHB production was Burk's buffer salts, 50 µM ferric citrate, 3% (w/v) glucose and 0.1% (w/v) fish peptone (Page, 1992). Cultures were incubated at high or low aeration (50 ml or 100 ml per 500 ml Erlenmeyer flask, respectively) with shaking at 225 r.p.m. at 28–30 °C. Growth curves were constructed from multiple flasks that were inoculated at the same time so that all the samples at a time point were removed from a single flask. Low-aeration conditions were also created for the incubation of Petri plates using a microaerophilic gas-generating kit (Oxoid CampyPak) in various sized jars to give a calculated 6, 11 and 16% (v/v) O₂.

Strain UWD cells for shift-down growth experiments were grown for 48 h in 100 ml Burk's medium, then harvested by centrifugation, washed in Burk's buffer and resuspended in an equal volume of Burk's buffer. The cell suspension was

dispensed 20 ml per 50 ml Erlenmeyer flask and incubated at 30 °C with shaking at 225 r.p.m. for up to 8 d.

Analysis of cells and culture fluids. Capsule was solubilized by the addition of 1 ml 5.0 M NaCl and 2 ml 0.05 M disodium EDTA, pH 7.0, to 50 ml culture prior to centrifugation at 10000 g (10 min) to remove the cells. Alginate in the culture supernatant fluid was precipitated with 3 vols ice-cold 95% (w/v) ethanol overnight at 4 °C. The precipitated alginate was collected on a Whatman GF/A glass fibre filter and dissolved in 0.1 M NaOH at room temperature prior to assay by the carbazole assay (Knutson & Jeanes, 1968) using commercial alginate (Sigma) as a standard. PHB dry weight was determined after digestion of cell material in commercial bleach (Law & Slepecky, 1961). Cell protein, glucose and ammonia remaining in the culture fluid were determined by colorimetric assays [the Lowry method, Trinder – Sigma Diagnostics, and Bergersen (1980), respectively]. Alkyl resorcinols were extracted from cell material with acetone overnight at room temperature (Kozubek & Tyman, 1995), then assayed by a colorimetric assay using Fast Blue B with orcinol as the standard (Tluscik *et al.*, 1981). β -Galactosidase specific activity (Miller, 1972) was measured as A_{420} (mg cell protein)⁻¹ h⁻¹, rather than optical density, because culture optical density was greatly affected by the PHB content of the cells. NADH oxidase activity was assayed as described previously (Page, 1991).

DNA isolation and PCR amplification of *algU*. Chromosomal DNA was extracted from *A. vinelandii* (Robson *et al.*, 1984). The region of *algU* expected to contain a natural insertion element (Martinez-Salazar *et al.*, 1996) was amplified by PCR. The Expand Long Template PCR System (Boehringer Mannheim) was used in buffer system 3 with 200 µM dNTPs, 1.25 µM of each primer, 1.5 mM MgCl₂, 400 ng chromosomal DNA, 5% (v/v) DMSO and 2.5 units of the DNA polymerase mix in a final volume of 100 µl. The primers for *algU* amplification were WJP13 (5'-ATGTTAAACCAGGAGCA-AGATCA-3') and WJP46 (5'-CATAAACTCGGCATCA-CCTGCA-3'). The PCR program (Minicycler, MJ Research) was: 5 min denaturation at 95 °C, followed by 30 cycles of 30 s denaturation at 95 °C, 1 min annealing at 37 °C, and 2 min extension at 68 °C.

Southern hybridization followed standard procedures (Southern, 1975), using ³²P-end-labelled WJP12 (5'-GGCTAT-GTCTGAGTTAGCTGTT-3') as a probe for the insertion element and WJP51 (5'-GAAATGCTGATACGCATTG-CGTTC-3') as a probe for *algD*.

RNA extraction and primer extension analysis. *A. vinelandii* cultures were grown for 24 and 48 h in Burk's medium prior to RNA extraction and primer extension analysis as described previously (Tindale *et al.*, 2000). The oligonucleotide WJP51 was used as the primer.

The sequence ladder used to identify the starts of *algD* transcription was derived from the strain UWD *algD* promoter region, which had been amplified by PCR using primers WJP50 and WJP49. Primer WJP50 hybridized with a sequence 389 nt upstream of the start of the *A. vinelandii* ATCC 9046 *algD* gene (Campos *et al.*, 1996) and had a *Pst*I site (lower case) tagged on for subsequent cloning of the PCR product: 5'-ctctctgcagATTACCTATTCCACACTGG-3'. The reverse primer WJP49 hybridized with a sequence 199 nt into the *algD* gene and had an *Eco*RI site (lower case) tagged on: 5'-tctcgaaattcGGGGATTTGCCCTGATTGAT-3'. The amplified strain UWD *algD* promoter region was found to have a nucleotide sequence identical to the *algD* promoter region of ATCC 9046 (GenBank U11240).

RESULTS AND DISCUSSION

Alginate formation by strain UWD

Under high-aeration conditions, strains UW and UWD formed $< 5 \mu\text{g}$ alginate $(\text{mg cell protein})^{-1}$ during 24 h incubation and at most $11 \mu\text{g}$ alginate $(\text{mg cell protein})^{-1}$

Fig. 1. Alginate production by strain UWD. Cultures were incubated under reduced aeration conditions and the biomass of strains UW (○) and UWD (□) (a), and PHB (●) and alginate production (○) (b) were assayed. (c) Alkyl resorcinol production (□) coincided with glucose limitation (■).

Fig. 2. Rates of alginate production in strains UWD and UW. Alginate production by strains UWD (■) and UW (●) was measured in cultures grown at reduced aeration. Best fit lines using mean values are shown. Duplicate cultures differed by $\pm 5 \mu\text{g}$ $(\text{mg cell protein})^{-1}$.

after 48 h incubation. By comparison, alginate formed by the capsule-positive, parent strain O was about $400 \mu\text{g}$ $(\text{mg cell protein})^{-1}$ after 24 h and at least 1 mg $(\text{mg cell protein})^{-1}$ after 48 h. However, significant amounts of alginate were formed by strain UWD under reduced aeration conditions. Increased alginate production was first observed at about 36 h at the onset of the stationary phase of growth and it continued for 4 d, yielding about $400 \mu\text{g}$ alginate $(\text{mg cell protein})^{-1}$ (Fig. 1a, b). The culture was ammonium-limited by 20 h (data not shown) and glucose-limited by 2 d (Fig. 1b). Thus a large amount of alginate was formed after glucose limitation.

The most likely source of carbon for continued alginate formation by strain UWD was PHB, which appeared to be turned over after 2 d (Fig. 1b). PHB was turned over at a rate of $400 \mu\text{g}$ $(\text{mg cell protein})^{-1} \text{ d}^{-1}$ ($r^2 = 0.945$) and the alginate production rate was $90 \mu\text{g}$ $(\text{mg cell protein})^{-1} \text{ d}^{-1}$ ($r^2 = 0.926$). Turnover of PHB was also indicated by the production of alkyl resorcinols, which are specifically formed by PHB catabolism (Reusch & Sadoff, 1981). Elevated levels of resorcinols coincided with exogenous glucose exhaustion (Fig. 1c) and increased alginate formation (Fig. 1b).

When alginate production by strain UWD was examined on a finer timescale, two rates of alginate formation occurred during the first 50 h of incubation (Fig. 2). During exponential growth (at times prior to 30 h, Fig. 1a), the alginate production rate was $1.2 \mu\text{g}$ $(\text{mg cell protein})^{-1} \text{ h}^{-1}$ ($r^2 = 0.969$). During stationary phase, the rate increased to $4.8 \mu\text{g}$ $(\text{mg cell protein})^{-1} \text{ h}^{-1}$ ($r^2 = 0.997$). This rate was similar in magnitude to the long-term rate of $3.8 \mu\text{g}$ $(\text{mg cell protein})^{-1} \text{ h}^{-1}$ observed in

Fig. 3. Alginates production by PHB-filled strain UWD cells. Cells, grown for 48 h in glucose medium, were harvested, washed and resuspended in Burk's buffer (without exogenous carbon source). (a) Alkyl resorcinol (●) and (b) alginate (□) and PHB (■) content of the cells was measured during 8 d further incubation.

Fig. 1(b). In contrast, strain UW formed alginate at a low but constant rate of $0.6 \mu\text{g (mg cell protein)}^{-1} \text{h}^{-1}$ ($r^2 = 0.951$) (Fig. 2). Strain O formed alginate at a much higher rate of $168 \mu\text{g (mg cell protein)}^{-1} \text{h}^{-1}$ ($r^2 = 0.887$) until stationary phase, when the rate decreased to $\approx 3 \mu\text{g (mg cell protein)}^{-1} \text{h}^{-1}$ ($r^2 = 0.031$), presumably due to O_2 limitation (data not shown).

The alginate formed by strain UWD was loosely associated with the cell and was released into the culture fluid. Centrifugation of these cultures (without prior NaCl and EDTA treatment) gave soft mucoid cell pellets characteristic of strain O. However, when strain UWD was sampled from a 48 h culture and streaked onto solid medium, the isolated colonies that formed were not mucoid like strain O, but had the typical butyrous morphology of strain UW, with the opaque white colour characteristic of strain UWD (Page & Knosp, 1989). Strain UWD also failed to form mucoid colonies when the plates were incubated for 1 week under reduced O_2 concentrations. Thus the production of alginate by strain UWD was not due to reversion to a capsule-positive genotype, but appeared to be a reversible, phenotypic change.

Fig. 4. Transcriptional activity of *algD::lacZ* fusion in strains DU5 and U5. β-Galactosidase activity in strains DU5 (●) and U5 (■) during incubation at reduced aeration is shown. Stationary phase occurred at about 30 h, as in Fig. 1(a). Best fit lines using mean values (duplicate cultures differed by ± 2.5 specific activity units) are shown.

PHB-filled cells form alginate

In order to confirm that alginate formed by strain UWD was derived from PHB turnover, cells that had been grown for 48 h in Burk's medium were used to inoculate Burk's buffer (without an exogenous carbon source) and incubated for up to 8 d. During the course of this nutrient shift-down, extracellular alginate rapidly increased while PHB decreased from 13.4 to $9.8 \text{ mg (mg cell protein)}^{-1}$ (Fig. 3b). Alkyl resorcinols also formed coincident with PHB turnover in the first 5 d of incubation (Fig. 3a). The rate of PHB degradation was $407 \mu\text{g (mg cell protein)}^{-1} \text{d}^{-1}$ ($r^2 = 0.740$) and the rate of alginate formation was $147 \mu\text{g (mg cell protein)}^{-1} \text{d}^{-1}$ ($r^2 = 0.854$).

PHB turnover will result in the formation of acetyl-CoA (Anderson & Dawes, 1990), with gluconeogenesis forming glyceraldehyde 3-phosphate at the branch point from the Entner-Doudoroff pathway to alginate production (Beale & Foster, 1996). On a carbon basis alone, $1.5 \text{ mol } \beta\text{-hydroxybutyrate (C}_4\text{)}$ would need to be consumed to generate $1 \text{ mol mannuronic acid (C}_6\text{)}$. Since the residual cell mass (cell dry weight minus PHB dry weight) did not change significantly during stationary phase (data not shown), the amount of PHB turned over in Figs 1 and 3 is more than sufficient to account for the alginate formation observed.

Inactivation of alginate formation in strain UWD

DNA from *A. vinelandii* strain U5 (*algD::TnSlacZ*) was transformed into strain UWD and Kan^R transformants were selected. These transformants (designated DU5) formed $\leq 5 \mu\text{g alginate (mg cell protein)}^{-1}$ under O_2 -

Fig. 5. Identification of the insertion element in the *algU* gene. (a) PCR was conducted using primers WJP13 and WJP46 and chromosomal DNA as the template. Products obtained with strains UWD (lane 1), O (lane 2), UW136 (lane 3), UW (lane 4) and 100 bp molecular mass standard (lane 5) were resolved on a 1.2% agarose gel. (b) Southern blot of gel (a) probed with the end-labelled insertion element probe. Arrow indicates 1000 bp standard.

limited growth conditions, suggesting that homologous recombination and inactivation of *algD* had occurred. This was confirmed by Southern hybridization of *NotI*-digested chromosomal DNA using WJP51 as a probe for *algD*. Strains UWD, U5 and DU5 produced a single restriction fragment that contained *algD*. The U5 and DU5 fragment was larger than that found in UWD, owing to the insertion of about 4 kb of Tn5lacZ DNA (de Lorenzo *et al.*, 1990) into *algD* (data not shown).

Under optimal PHB production conditions, strain DU5 produced 8.0 ± 0.2 mg PHB (mg cell protein)⁻¹ like strain UWD, which was four times that formed in strain UW. Strains DU5 and UWD formed PHB from glucose at maximum theoretical efficiency (0.33 ± 0.3 g g⁻¹) (Collins, 1987) since alginate formation occurred after PHB accumulation (as in Fig. 1b). Upon glucose limitation, strain DU5 turned-over PHB and formed alkyl resorcinols, but as noted above alginate was not formed.

Transcription of *algD* was examined in strains U5 and DU5 to determine if there was an obvious increase in *algD* transcription at the times when alginate formation was active (Fig. 4). The rates of *algD* transcriptional activity were not significantly different in strains DU5 and U5 during the exponential phase of growth. This activity reached a plateau in strain U5 as the cells entered the stationary phase, but continued unabated in strain DU5 (Fig. 4). The amount of LacZ activity in these cells was very low, suggesting that *algD* transcription was initiated from a weak promoter.

Strain UWD has an inactive *algU*

Since strain UWD was constructed by transformation with DNA from the alginate-positive strain 113, we questioned whether *algU* was still inactive in strain UWD. The region of the *algU* gene expected to contain the natural insertion element was amplified by PCR, using primers WJP13 (hybridizing to the 5' end of *algU*)

and WJP46 (hybridizing to *algU* downstream of the insertion element). The expected PCR products with and without the insertion element were about 1322 and 322 bp, respectively (calculated from Martinez-Salazar *et al.*, 1996). As shown in Fig. 5(a), there was a prominent PCR product formed in strains UWD, UW136 and UW that was just greater than 1300 bp and a much smaller PCR product was formed in strain O at ~300 bp. Only the larger PCR product hybridized with oligonucleotide WJP12 (Fig. 5b), which was complementary to the insertion element sequence. Thus strain UWD had an inactive *algU* gene as observed in strains UW and UW136.

Transcription of *algD* in strain UWD

Primer extension was used to determine the start of *algD* transcription in strains O and UWD after 24 and 48 h incubation. In strain O, transcription of *algD* was initiated at a G (P2 in Fig. 6a, b) downstream from the AlgU promoter. This differs from that reported by Campos *et al.* (1996), who show the start site as the next C upstream, but their published figures are of poor quality. Transcription from P2 was not active in strain UWD (Fig. 6b), consistent with the absence of a functional *algU* gene (Fig. 5) and suggesting that an alternative RpoE sigma factor is not available in strain UWD to activate transcription from this promoter.

The other known start of transcription (Campos *et al.*, 1996), designated P1, was active in both strains O and UWD (Fig. 6a, c). Although primer extension is not strictly quantitative, there did appear to be an increase in the use of the P1 start site in strain UWD at 48 h, while there was no increase in the use of any of the start sites in strain O at 48 h. A third site of unknown function (Campos *et al.*, 1996) and designated P3 (Fig. 6a) was evident in strains O and UWD at both times (data not shown). Further description of this site, which is not likely to be a true transcription start site, will appear elsewhere (unpublished data).

Fig. 6. Primer extension analysis of *algD* transcription in strains O and UWD. (a) Sequence of the *algD* upstream region, including the AlgU promoter and P2 transcription start site, RpoD promoter and P1 transcription start site, the P3 site, ribosome-binding site (rbs) and translation start site (ATG). RNA was prepared from strain O after 24 h (lane 1) and 48 h (lane 2) and UWD after 24 h (lane 3) and 48 h (lane 4) incubation under low-aeration incubation conditions. Starts of transcription are shown by arrows at P2 (b) and P1 (c). The sequence ladder was generated by reverse transcription from primer WJP51.

Table 1. Characteristics of strains UW, UWD and DC

Analysed after 48 h incubation under low-aeration conditions, using at least duplicate assays. Values are means $\pm$ range.

Strain	NADH oxidase specific activity*	PHB [mg (mg cell protein) ⁻¹]	Alkyl resorcinol [μg (mg cell protein) ⁻¹]	Alginate [μg (mg cell protein) ⁻¹]
UW	0.71 $\pm$ 0.15	1.53 $\pm$ 0.5	0.0 $\pm$ 0.0	3.4 $\pm$ 3.0
UWD	0.16 $\pm$ 0.05	3.81 $\pm$ 0.5	63.0 $\pm$ 3.0	320.0 $\pm$ 10.8
DC†	0.68 $\pm$ 0.10	1.87 $\pm$ 0.5	0.2 $\pm$ 0.5	3.7 $\pm$ 1.7

* μmol NADH oxidized min⁻¹ (mg membrane protein)⁻¹.

† Mean values from analysis of 10 individual transformants.

Growth-phase-dependent alginate formation

Since strains UW and UWD are identical in terms of having an inactive *algU*, the difference in alginate formation may be a consequence of the inactive respiratory NADH oxidase in strain UWD (Page & Knosp, 1989). To test this, strain UWD was backcrossed with strain UW DNA and less opaque (clear) colonies were picked. These colonies were not observed in strain UWD culture, but occurred at a frequency of 3×10^{-2} in transformed culture. Ten transformants (designated DC) were analysed and found to have increased NADH oxidase activity compared to strain UWD, lower PHB yields and had minimal alginate production like strain UW (Table 1). However, strain DC was rifampicin-

resistant like strain UWD, which distinguished the transformants from the donor strain UW, which was rifampicin-sensitive (Page & Knosp, 1989).

Normally PHB is formed in the stationary phase of growth, when the culture is O₂-limited or deprived of an essential nutrient other than the carbon source (Anderson & Dawes, 1990). The abundance of NADH at the transition to stationary phase is a trigger for PHB formation (Anderson & Dawes, 1990). Also, the NADH/NAD ratio is a well-known regulator of catabolism (Neidhardt *et al.*, 1990) and the redox state of a cell is a powerful regulator of gene expression (Bauer *et al.*, 1999). The hyperproduction of alginate in strain UWD in stationary phase (Figs 1 and 2) is probably a

reflection of increased availability of PHB for turnover. However, the increased activity of *algD* transcription (Fig. 4) probably also reflects altered promoter activity, rather than substrate availability per se. The RpoD promoter (P1) of *algD* must be important in this process, as it is the only recognized promoter that is active in strain UWD. It is possible that the RpoD promoter is also regulated by the stationary phase sigma factor RpoS, which is highly homologous to RpoD and frequently shares the same promoters (Espinosa-Urgel *et al.*, 1996; Hengge-Aronis, 1996). Promoters with a -10 sequence similar to CTATACT and with intrinsic upstream DNA curvature would be preferentially recognized by RpoS (Espinosa-Urgel & Tormo, 1993; Espinosa-Urgel *et al.*, 1996). The *algD* P1 promoter has the -10 sequence CTATAAT (Fig. 6a) and calculations made with DNASTAR software, as described by Espinosa-Urgel & Tormo (1993), show that the sequence upstream of P1 has intrinsic DNA curvature (data not shown). In support of this hypothesis, it has been shown that *algD* transcription and alginate formation in *A. vinelandii* is regulated by GacSA, a sensor kinase that influences RpoS levels in the cell (Castaneda *et al.*, 2000). Similarly, alginate formation in *P. aeruginosa* is greatly reduced in an RpoS mutant (Suh *et al.*, 1999). Our current research will determine the role of RpoS in PHB turnover and alginate biosynthesis in *A. vinelandii* UWD.

NOTE ADDED IN PROOF

The complete sequence of the *A. vinelandii* UW insertion element in *algU* has been deposited as GenBank accession number AF322366 (D. Meakins, A. Tindale & W. J. Page, Nov. 19, 2000).

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