Genetics of Bacterial Alginate: Alginate Genes Distribution, Organization and Biosynthesis in Bacteria

Muhammadi and Nuzhat Ahmed*

Centre for Molecular Genetics, University of Karachi, Karachi-75270, Pakistan

Abstract: Bacterial alginate genes are chromosomal and fairly widespread among rRNA homology group I *Pseudomonads* and *Azotobacter*. In both genera, the genetic pathway of alginate biosynthesis is mostly similar and the identified genes are identically organized into biosynthetic, regulatory and genetic switching clusters. In spite of these similarities, still there are transcriptional and functional variations between *P. aeruginosa* and *A. vinelandii*. In *P. aeruginosa* all biosynthetic genes except *algC* transcribe in polycistronic manner under the control of *algD* promoter while in *A. vinelandii*, these are organized into many transcriptional units. Of these, *algA* and *algC* are transcribed each from two different and *algD* from three different promoters. Unlike *P. aeruginosa*, the promoters of these transcriptional units except one of *algC* and *algD* are *algT*-independent. Both bacterial species carry homologous *algG* gene for Ca^{2+} -independent epimerization. But besides *algG*, *A. vinelandii* also has *algE1-7* genes which encode C-5-epimerases involved in the complex steps of Ca^{2+} -dependent epimerization. A hierarchy of alginate genes expression under $\sigma^{22}(\text{algT})$ control exists in *P. aeruginosa* where *algT* is required for transcription of the response regulators *algB* and *algR*, which in turn are necessary for expression of *algD* and its downstream biosynthetic genes. Although *algTmucABCD* genes cluster play similar regulatory roles in both *P. aeruginosa* and *A. vinelandii* but unlike, transcription of *A. vinelandii*, *algR* is independent of σ^{22} . These differences could be due to the fact that in *A. vinelandii* alginate plays a role as an integrated part in desiccation-resistant cyst which is not found in *P. aeruginosa*.

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INTRODUCTION

Bacterial alginate is a linear exopolysaccharide consisting of β -1,4-linked β -D-mannuronic acid and its C-5 epimer α -L-guluronic acid [15,36,75,50]. Bacterial alginate is partially acetylated at the O-2 and/or O-3 positions of mannuronic acid residues [43-46,127]. Alginate polymer is primarily synthesized as polymannuronate [51,86,127] and monomers distribution is variable throughout the polymer due to epimerization of some of mannuronic acid residues [124,127]. Only two bacterial genera *Pseudomonas* [51,75] and *Azotobacter* [41,106] are known to produce alginate. In *P. aeruginosa* alginate acts as an extracellular matrix material that allows the formation of differentiated biofilms, which restrict diffusion of clinical antibiotics and protect embedded cells against human antibacterial defense mechanisms [78,96]. Whereas alginate produced by phytopathogenic *Pseudomonads* is a virulence factor providing the encapsulated bacteria with a protective environment and being responsible for the water-soaked symptoms in infected plant tissue [116,150]. Under adverse environmental conditions *A. vinelandii* produces alginate both as a vegetative state capsule and as an integrated part of a particular resting stage form (desiccation-resistant cyst) [103,117]. Although the major aspects of the molecular genetics of alginate biosynthesis in *A. vinelandii* have also been reported [112] but most

of our knowledge of the genetics of alginate biosynthesis originates from extensive studies of *P. aeruginosa* [110,111, 147]. Mainly because of the medical relevance of this organism as an opportunistic human pathogen that causes severe and life-threatening infections in immuno compromised hosts such as patients with respiratory diseases, burns, cancers undergoing chemotherapy, and cystic fibrosis [66,112, 147]. Alginate plays an important role as a virulence factor during the infectious [50]. Therefore, *P. aeruginosa* has been a model for genetic studies of bacterial alginate biosynthesis. The genetics of bacterial alginate synthesis was first studied by the ability to complement a series of *Alg*⁻ (alginate non-producer/nonmucoid) mutants from stable *Alg*⁺ (alginate producer/mucoid) *P. aeruginosa* 8830 [19]. All *P. aeruginosa* strains carry the genes that encode the regulatory and biosynthetic machinery for alginate [137] and much knowledge on the genetic regulation of alginate biosynthesis has emerged during last two decades [51,53,112,147]. The aim of the present study is to review the independent studies and progress in molecular genetics of alginate biosynthesis and regulation in bacteria.

DISTRIBUTION OF ALGINATE GENES

Alginate (*alg*) genes are distributed throughout the *Pseudomonas* rRNA homology group I-*Azotobacter*-*Azomonas* lineage [39,41], while only some *alg* genes have been retained in the *Pseudomonas* group V (*Xanthomonas*) and enteric lineages but could not produce alginate [41]. Bacterial species belonging to *Pseudomonas* rRNA homology group I

*Address correspondence to this author at the Centre for Molecular Genetics, University of Karachi, Karachi-75270, Pakistan; Tel/Fax: +92-21-4966045; E-mail: a_nuzhat@yahoo.com

are actually more closely related to enteric bacteria (*E. coli*, *K. pneumoniae*) than to *Pseudomonas* species belonging to *Pseudomonas* rRNA homology groups II, III, and IV [142]. Probes for three alginate structural genes (*algA*, *algC* and *algD*) and one regulatory gene *algR(algR1)* of *P. aeruginosa* hybridize to DNA fragments from *P. syringae* pv. *glycinea*, *P. viridiflava* and *P. corrugate* [39] and other members of rRNA homology group I [41]. Genomic DNA from representatives of groups I-IV gave very weak or no hybridization with the probes, except for *algC*, indicating that the ability to produce alginate is restricted to members of rRNA homology group I. This substantiates earlier physiological studies in which alginate was isolated from *P. fluorescens*, *P. putida*, *P. mendocina* [59] and *P. syringae* [38,62]. *Azomonas* and *Azotobacter* species are closely related to *Pseudomonas* group I [11,29]. *A. vinelandii* possessed sequences homologous to all the *P. aeruginosa* *alg* genes tested, while *Azomonas macrocytogenes* lacked only *algD* [41]. It is not known whether *Azomonas* species produce alginate. While the alginate-specific genes (*algD* and *algR*) would be restricted to group I *Pseudomonas* species or other organisms known to produce alginate [31,39]. In addition, naturally occurring alginate-producing strains of plant-associated *P. fluorescens* were also reported [37]. But certain phytopathogenic fluorescent *Pseudomonas* species are occasionally found to produce alginate both in plants and *in vitro* [37,38]. All the phytopathogenic *Pseudomonads* tested (both Alg⁺ and Alg⁻) possess genetic sequences homologous to the *P. aeruginosa* alginate genes. Thus, it appears that many *Pseudomonas* species are capable of producing alginate but the genes involved in alginate biosynthesis are not normally expressed [41]. However, it is difficult to be precise about the number of genes that are solely associated with alginate biosynthesis as it is now clear, for example, that some of the control genes act globally and encode proteins such as *algA* and *algC* are also essential for lipopolysaccharide (LPS) biosynthesis [58]. Similarly, homology searches of *algI*, *algJ*, and *algF* amino acid sequences suggested that these genes encode a family of proteins involved in the esterification of surface or extracellular polysaccharides in a variety of bacteria have evolved by lateral gene transfer [46]. Since many of the *P. aeruginosa* alginate genes have been cloned, it is now possible to examine genomic DNA from various *Pseudomonas* species and other organisms phylogenetically near *P. aeruginosa* for sequences homologous to the *P. aeruginosa* *alg* genes to determine the extent to which the presence of *alg* gene sequences parallels phylogenetic relationships.

ORGANIZATION OF ALGINATE GENES

Irrespective of sequence homology, the physical order and organization of *alg* genes in *A. vinelandii* and alginate producing *Pseudomonas* species have been described identical to those of *P. aeruginosa* [1,72,77,90,105,110]. To date, more than 25 *alg* genes some of these with alternative names have been identified in *P. aeruginosa* and these alginate genes are all located on the bacterial chromosome, with no evidence of plasmid involvement [51,60,96,112] (Table 1, Fig. 1). In *P. aeruginosa*, the genes that encode the biosynthesis and regulation of alginate map to five distinct chromosomal loci [1,51,57,76,96,148] (Fig. 1). Based on their function, *alg* genes in *P. aeruginosa* are organized into three

clusters namely, structural/biosynthetic, regulatory and genetic switching genes (Table 1). Virtually all of the biosynthetic genes including *algD*, *alg8*, *alg44*, *algK*, *algE(alg76)*, *algG*, *algX(alg60)*, *algL*, *algI*, *algJ*, *algF*, *algA* and *algC* directly catalyzing the synthesis of alginate are clustered within an 18kb region located at 34-min on the *P. aeruginosa* chromosome except *algC* which is located at 10-min along with a cluster of alginate regulatory genes [1,1,6,51,80,110,118] (Fig. 1). Twelve structural genes located at 34-min are organized and transcribed as an operon with transcriptional initiating at the *algD* promoter, the proximal gene of the alginate biosynthesis operon [16,21,45,51,76,111] (Fig. 1). Whereas *algC* at 10-min transcribes in same direction but under the control of its own promoter region [151,152]. Five genes control the regulation of alginate production include *algZ(fimS)*, *algR(algR1)*, *algQ(algR2)*, *algP(algR3)* and *algB* [61,76,125]. All regulatory genes are located at 9-min except *algB* which is located at 13-min on chromosome [15,21,28,56,96] (Fig. 1). *algR* is located just immediately downstream of *algZ* [149] and 4.5kb apart from *algQ*. *algQ* and *algP* are linked but not immediately adjacent [25,91]. All regulatory genes have same transcriptional orientation except *algB* which transcribes in the direction same as biosynthetic gene cluster [111] (Fig. 1). The alginate switching genes which mediate the conversion to constitutive Alg⁺ phenotype, consisting of five genes, *algT(algU)*, *mucA(algS)*, *mucB(algN)*, *mucC(algM)* and *mucD(algY/algW)* are clustered forming part of the same transcriptional unit at 68-min on *P. aeruginosa* chromosome [15,51,76,83,85,87] (Fig. 1). Schlichtman and coworkers have also studied the one more alginate regulatory gene *algH* but its location on chromosome is still unidentified [119].

GENETIC REGULATION OF ALGINATE BIOSYNTHESIS

The regulation of alginate biosynthesis is complex and involves specific gene products and those that act globally [51]. The transcriptional activation of *algD* gene is key point in pathway regulation of alginate synthesis and is mediated by alginate switching and regulatory genes [13,23]. Alginate gene expression is transcriptionally controlled by *algT(algU)*-*mucA*-*mucB(algN)*-*mucC(algM)*-*mucD(algW/Y)* clustered at 68-min on the chromosome [85,144,146] (Fig. 1). These five genes cluster comprise the main genetic switch, controlling the conversion between non-mucoid to mucoid forms of *P. aeruginosa*. The *algT* gene encodes a 22kDa alternative sigma factor (σ^{22}) with significant similarity to the alternative RNA polymerase sigma factor σ^E from *Escherichia coli* and positively regulates its own promoter (*PalgT*) as well as the promoters of *algR*, *algB*, *algD*, and *algC* [63,85,122,125] (Fig. 1). This is because, the promoter regions preceding the transcriptional initiation sites of *algT*, *algR*, *algB*, *algD* and *algC* all have significant homology to promoters recognized by σ^E [33,63,83,102]. Actually, $\sigma^{22}(\text{algU})$ forms a complex with RNA polymerase [76,96,122] (Fig. 1). This RNAP- σ^{22} complex positively regulates the transcription of *algD* and *algR* because promoters of both genes have consensus sequences for complex binding at the -35/10 region which is consistent with recognition by σ^{22} [30,90,120]. The other genes in the *algT* cluster appear to regulate the expression or activity of σ^{22} . *mucA* encodes an

Table 1. Alginate Genes Clusters, Size, Mapping Sites and Products in *P. aeruginosa*

Gene cluster	Gene	Size (bp)	Gene product	References
Structural/biosynthetic	algD	1311	GDP-mannose dehydrogenase	[23,127,135]
	alg8	1494	subunit of alginate polymerase	[79,140]
	alg44	1170	subunit of alginate polymerase	[79,140,141]
	algK	1428	subunit of protein scaffold	[64,132]
	algE(alg76)	1473	porin-like OM protein	[17,107, 148]
	algG	1632	mannuronan C-5 epimerase	[15,44,128]
	algX(alg60)	1425	subunit of protein scaffold	[95,112,141]
	algL	1104	alginate lyase	[10,95,117,141]
	algI	1563	acetylase	[44,46,110]
	algJ	1176	acetylase	[44,46,110]
	algF	651	acetylase	[42,45,125]
	algA	1446	PMI-GMP	[20, 50,148]
	algC	1636	PMM	[152,153]
Regulatory	algZ(fimS)	1077	RHH DNA-binding protein	[4,5,150]
	algR(algR1)	747	response regulator protein of TCSTS	[24,25, 68]
	algQ (algR2)	483	cognate sensor Kinase	[66,69, 114,118]
	algP(algR3)	1058	histone-like protein	[67,70,148]
	algB	1350	NtrC subclass of TCSTS	[55, 56,144]
Genotypic switching	algT(algU)	582	sigma factor σ^{22}	[51, 84,121]
	mucA(algS)	585	anti- σ^{22} factor	[80,84,121,122]
	mucB(algN)	951	anti- σ^{22} factor	[80,84,121,122]
	mucC(algM)	456	homologue of PhoORF4 product	[7,8]
	mucD(algW/Y)	1425	homologue of serine protease (HtrA)	[7, 51,148]

OM(outer membrane), PMI-GMP(phosphomannose isomerase-/guanosinediphosphomannose pyrophosphorylase), PMM(phosphomannomutase), **PhoORF4**(*Photobacterium* sp SS9 ORF4).

anti- σ^{22} factor (Table 1) with affinity for σ^{22} and is a negative regulator of *algT* transcription [83,118,120] (Fig. 1). *mucB* also encodes an indirect anti- σ^{22} (Table 1, Fig. 1) which interacts with periplasmic domain of *mucA*, thereby altering its conformation so that it binds σ^{22} and target it for degradation [62,85,122,146]. *mucD* encodes a periplasmic protease appears to act as a negative regulator of σ^{22} (Fig. 1) and has homology to the HtrA(DegP) heat shock periplasmic protease of *E. coli* [7]. Whereas the product of *mucC* is homologous to that of *Photobacterium* sp. strain SS9 which function as negative [7,8] (Fig. 1) or alternatively as positive and negative regulator [102] but like *mucB*, *mucC* also does not directly affect *algU* activity [8]. Inhibition of σ^{22} activity by *muc* genes results in a nonmucoid phenotype. Spontaneous mutations in *mucA*, *mucB* and *mucD* represent the major mechanism of conversion from the nonmucoid to the mucoid phenotype [7,8,85]. However, these mutations are unstable and spontaneous reversion to the Alg⁻ phenotype often occurs

due to suppressor mutations in *algT* [33,121,122]. Further, inactivation of the *muc* genes results in a high-level transcription of alginate biosynthetic and regulatory genes, such as *algD* and *algR* respectively, leading to the mucoid phenotype [7,26,81,82]. *algT* might not be the only sigma factor that is responsible for *algD* transcription. Under certain conditions, transcription of *algD* requires RpoN(σ^{54}). RpoN-dependent expression of *algD* is only observed in a *muc23* background, where mutations are found in currently uncharacterized gene(s) that map to a chromosomal locus distinct from *mucA* [9]. Besides the control exerted by genetic switching genes, the regulatory genes located at 13- and 10-min are also required for high level alginate production through the activation of *algD* transcription (Fig. 1). The regulatory genes *algR*, *algB*, *algQ*, *algZ* and *algP* express some sort of proteins which act primarily through DNA binding and bending similar to the members of bacterial two-component signal transduction systems (TCSTS) [27,28,143]

(Table 1). The best characterized regulatory gene *algR* transcribes in response to protein σ^{22} and encodes response regulator protein of TCSTS which functions as positive transcriptional regulator of genes such as *algD* [24,64,146], *algR* [69,70,96], *algC* [151,152] (Fig. 1) and the neuraminidase gene *nanA* [12]. In conjunction with *algU*, *algR* up-regulates transcription of *algD* and the downstream structural genes [51] (Fig. 1). *algQ* encodes 18kDa *cognate sensor Kinase* (Table 1) that undergoes autophosphorylation in the presence of ATP or GTP and transfers the acquired phosphate to *algR* [25,71,114,115] (Fig. 1). Functional *algQ* is important for *algR*-mediated transcriptional activation of the *algD* promoter [67,71,152]. *algR* binds three regions (named RBS) within the *algD* promoter at 14-mer sites having the sequence CCGTTCGTCN5' and located upstream of the *algD* transcriptional initiation site [69,91,94] (Fig. 1). The first two sites are located far upstream of the *algD* transcriptional initiation site, at nucleotide positions -465 to -452 and -389 to -376, and have been shown to be critical for high-level expression of *algD* [69,130,121]. *algR* has also been shown to bind weakly with the *algD* promoter region between positions -144 and +11 [93]. There are also two sites for the binding of integration host factor (IHF) protein [138,145] as well as *CysB* and *E. coli* CAP binding sites [22,31] (Fig. 1). Mutagenesis studies suggest that both IHF and *CysB* play a role in *algD* activation, albeit much reduced in comparison with *algR* [22,145]. Another key alginate regulatory gene called *algB* is essential for high-level synthesis of alginate in *P. aeruginosa* [56]. Like *algR*, *algB* belongs to the response regulator superfamily of TCSTS. *algB* encodes a protein NtrC subclass of TCSTS (Table 1) with a molecular size of 49kDa and is required for the transcriptional activation of *algD* [143,145] (Fig. 1). Transcription of *algB* requires both the product of the *algT* gene and the DNA binding-bending protein IHF [144] (Fig. 1). *algZ* gene encodes for an absolutely σ^{22} -dependent, ribbon-helix-helix (RHH) DNA binding protein proposed to be the *cognate sensor kinase* which binds to sequences located 280bp upstream of the *algD* promoter and activates the *algD* transcription [4,5,149]. *algP* encodes histone-like protein which plays a role in alginate production and might contribute to DNA looping [61] (Fig. 1). An *algR* mutation was constructed in FRD1, and this resulted in the loss of alginate production and a dramatic decrease in *algD* transcription. RNA and gene fusion analysis revealed that *algB* is not required for *algR* expression, nor is *algR* necessary for transcription of *algB*. Thus, with the exception of a requirement for *algT*, the *algB* and *algR* pathways appear to be independent of each other [146]. Mutation in *algT* blocks expression of *PalgD* and reduces expression of the alginate regulatory genes *algR* and *algB* [144,146]. *algT* or a protein under *algT* control also binds to sequences located within the *algD* promoter. Expression of the alginate biosynthetic operon requires both the response regulators (*algB* and *algR*) and *algT* (Fig. 1). Expression of *algB* and *algR* is dependent on *algT*, suggesting a circuit in which *algT* activates *algB* and *algR* [146]. Several proteins have been shown to bind *algD* sequences and are required for full transcriptional activation. As all of these genes are required, but none appear to be sufficient for full *algD* activation, this suggests a complex three-dimensional nucleoid structure for this promoter [5]. *algC* gene encodes a bifunctional enzyme with both *phosphomannomutase* and *phosphoglucumutase*

activity [18,148] (Table 1) that is required not only for alginate production [148] but also for rhamnolipid production [103] and lipopolysaccharide expression [18,148]. Expression of *algC* is independent of other alginate genes yet *algC* is under the positive control of the response regulator *algR* in a similar manner as in case of *algD* [47,151] (Fig. 1). Transcriptional fusion studies of the *algC* promoter have shown that *algC* expression is reduced approximately fivefold in the absence of *algR* [151]. *algR* binds three regions positioned upstream and downstream of the transcription start site [47,130,131,151] yet the orientation and positioning of the *algR* binding sites differ in the *algC* and *algD* promoters. The differences in orientation of the *algR* binding sites and of the *algR* binding affinities between the *algC* and *algD* promoters and their effects on the mechanism of *algR* regulation *in vivo* have not been clearly established [131]. *algR* specifically bound with two regions of *algC* upstream DNA. A fragment spanning nucleotide positions -378 to -73 showed strong specific binding, while a fragment located between positions -73 and +187 interacted relatively weakly with *algR* [151]. Hence, *algR* regulates alginate production through binding to *algD* and *algC* promoters and interacts with RNA polymerase associated with *algU* [47,69,92-95,151,152] and causes a looping of promoters that is required for transcriptional activation [95].

ENVIRONMENTAL ACTIVATION

An important feature of alginate production by *P. aeruginosa* is that alginate genes are normally silent [51,57,137] but environmental stimuli like starvation, desiccation, dehydration, increasing osmolarity and the presence of phosphorylcholine activate a cascade of regulatory proteins involved in the activation of *algD* promoter [6,50,135,136,152] (Fig. 1). Further the *algD* promoter region shows homology to a number of bacterial promoters that are induced in response to environmental stress [136]. These include the osmoregulated promoters for *E. coli* outer membrane porin protein *OmpF* and *OmpC* [6,67]. In addition, the *algD* promoter has been found to contain a σ^{54} recognition sequences [70,136]. Further more, the presence of sucrose, NaCl and KCl in the medium also activate the *algD* transcription [136] (Fig. 1). However, there is also a report that glucose can stimulate the *algD* transcription and alginate production [79]. Similarly, phosphate and nitrogen limitation conditions have also been found to activate *algD* in similar fashion [30-32,136] (Fig. 1). Transcriptional activation of *algD* promoter, the first gene in operon of structural genes, is associated with conversion to Alg⁺ phenotype. In both Alg⁺ and Alg⁻ *P. aeruginosa* strains, the *algC* promoter, like the *algD* promoter [6], is also activated in a hyperosmotic environment [152]. This osmolarity-induced activation is found to be dependent on *algR* [131,151,152]. It has been reported that the mRNA levels of *algA*, *algC* and *algD* genes increased, coordinately, in the cells of mucoid *P. aeruginosa* grown under increasing dissolved oxygen tensions of up to 70% air saturation [74]. This environmentally induced transcription of the alg genes cluster and the resulting production of alginate occur only at a low level. Copious amounts of alginate are only produced in combination with inactivation (mutations) of the negative regulators of the *algU* activity such as *mucA*, *mucB*, *mucC* and *mucD* [7,8,81,82,85].

Fig. (1). Model of the regulation of alginate biosynthesis and physical map (unshaded pentagons along with circle) of alginate structural/biosynthetic (10/34-min), regulatory (9/13-min) and genetic switching (68-min), genes at chromosome in *Pseudomonas aeruginosa*. Heads of pentagons indicate direction of transcription and shaded shapes in side the circular chromosome are the gene products. CSK(cognate sensor Kinase), RRP(response regulatory protein), CAP(catabolite activator protein), + and - (positive and negative effect).

ALGINATE BIOSYNTHESIS

The alginate biosynthesis pathway can be divided into four different stages: (i) synthesis of precursor substrate in the cytoplasm [125], (ii) polymerization and cytoplasmic membrane transfer, (iii) periplasmic modification, and (iv) export through the outer membrane [129,139]. The precursors synthesis which occurs in the cytoplasm is well characterized and starts from the central metabolite fructose-6-phosphate, which is converted to active precursor GDP-mannuronic acid through a series of four enzymatic steps involving the proteins encoded by *algD*, *algA* and *algC* genes [40,44,73,74,86,108,113,125,128,147] (Fig. 2). Fructose-6-phosphate is first isomerized into mannose-6-phosphate by *phosphomannose isomerase* encoded by *algA*. Mannose-6-phosphate is then converted into mannose-1-

phosphate by *phosphomannomutase* encoded by *algC* [108, 148] (Table 1 and 2, Fig. 2). Then GDP-mannose is formed by the action of another enzyme *GDP-mannose pyrophosphorylase* encoded by *algA* gene [20,51,111,147] (Fig. 2). Finally, GDP-mannose is oxidized to GDP-mannuronate by *GDP-mannose dehydrogenase* encoded by *algD* [14,87,120, 128,134] (Table 1 and 2, Fig. 2). This last step is nonreversible and thought to be specific for alginate synthesis [40]. Although the polymerization steps are not well characterized but structural genes *alg8* and *alg44* are found to encode the catalytic subunits of the alginate polymerase localized in inner membrane which have sequence similarity to β -glycosyltransferase [80,139,140] (Table 1 and 2). The polymerization process occurs though these proteins localized in the periplasm [64,88]. The initial polymeric product of alginate biosynthesis pathway is polymannuronate and it has been

proposed that polymerization and secretion across the inner-membrane are accomplished simultaneously [87]. To produce finally biologically active structure, polymannuronate chain undergoes a series of post-polymerization modification steps carried out by a number of periplasmic proteins [40,113] (Fig. 2). Alginate is excised enzymatically by *algL* gene product alginate lyase from biosynthetic complex, which cleave the 1-4 glycosidic linkage by β -elimination, resulting an unsaturated nonreducing terminus [48,49,96, 118] (Table 1 and 2, Fig. 2). Although *algL* is required for normal production of alginate, may not be essential for polymerization in alginate synthesis [10,118] but it has been hypothesized that the alginate lyase may function either as an editing protein to control the length of the alginate polymer or to provide short pieces of alginate to prime the polymerization reaction [87]. The three structural genes *algI*, *algJ*, and *algF* encode a protein with seven transmembrane domains, a type II membrane protein, and a periplasmic protein respectively which form an enzyme complex (*acetylase*) (Table 1 and 2) that catalyzes the partial O-acetylation of polymeric mannuronic acid residues at the O-2 and/or O-3 positions [1,42,44,45,126] occurs either associated with the bacterial inner membrane or in the periplasm after polymannuronate polymerization [46]. *algG* encodes periplasmic

mannuronan C-5 epimerase (Table 1 and 2) which is required for epimerization of some of polymeric mannuronates to guluronic acid residues in the periplasm [15,43,129] (Fig. 2). But the *algG* mutants of *P. aeruginosa* were found to produce alginate containing only polymeric D-mannuronic acid residues [15,53]. *algK* gene encodes a 52kDa hydrophilic protein with apparent signal peptide sequence characteristic of a lipoprotein in periplasmic subcellular localization [64] while *algX* encodes a hydrophilic periplasmic protein with no extensive hydrophobic domains [113] (Table 1). Recent advances indicate that a periplasmic protein complex termed as protein scaffold is formed by direct interaction of C-5 epimerase with the product of *algK*, and *algX*. This protein scaffold surrounds and protects newly formed polymers from degradation by alginate lyase as they are transported within the periplasm for further modification and eventual transport out of the cell [53,64,65,113,129,147] (Fig. 2). *algE* gene located between *algK* and *algG* (Fig. 1) encodes an anionic selective porin-like outer membrane protein (Table 1) which forms an alginate-specific outer membrane channel through which alginate is exported across the outer membrane into medium environment [17,44,45,108,112] (Fig. 2).

Fig. (2). Model for the biosynthesis and assembly of alginate in *P. aeruginosa* [147]. I.M. and O.M.(inner and outer membrane respectively), G and M(guluronate and mannuronate respectively) and Ac(acetyl group).

Alginate Genetics in *A. vinelandii*

In *A. vinelandii* our understanding of the genetics of alginate synthesis has improved greatly over the last few years but the genetic regulation has remained largely unexplored. Although, in *A. vinelandii* the organization of identified alginate genes and their control the alginate biosynthesis are almost in a manner similar to those of the *P. aeruginosa* homologs [51,72,81,90,110] but some of genes in *A. vinelandii* still differ in their transcriptional organization, regulation and function from *P. aeruginosa* (Table 1 and 2). This is most probably because *A. vinelandii* produces alginate both as a vegetative state capsule and as an integrated part of a particular resting stage form (cyst) of this organism [118]. Alginate biosynthetic genes cluster in *A. vinelandii* similar to that described in *P. aeruginosa* [13,77,88,89,110] is organized in at least four transcriptional units *PalgD* [13,88,89], *Palg8-*alg44-*algK*** [88,89], *PalgG-X-L-V-I-F-A* and *PalgA* [141] (Table 2). However, transcription of *algA* exclusively from promoter upstream *algA* is not sufficient for the *algA* levels required for alginate production. The later three promoters are different from the *algD* promoter and do not depend upon *algT* and *algD* for their activation [77,84,88,89,141]. *algD* is transcribed from three promoters only one of which is indirectly *algT* dependent [97,101]. However, *algD* expression from the two *algT*-independent promoters is sufficient to account for alginate production [97]. But *algT* mutants are completely abrogated in alginate production [84,97], presumably due to the σ^E dependence of other genes involved in exopolysaccharide synthesis such as *algC* [52]. In contrast to *P. aeruginosa*, *A. vinelandii* does not require *algR* for activation of *algD* transcription from any of its promoters. Partial complementation of *P. aeruginosa* *algR* mutants with the *A. vinelandii* *algR* gene, however, implies that *A. vinelandii* *algR* can bind the RBS sequences present upstream of the *P. aeruginosa* *algD* promoter and can interact with *algU* RNA polymerase. This finding is consistent with the lack in *A. vinelandii* of sequences homologous to *P. aeruginosa* RBS [13]. It was proposed that a transcriptional regulator other than *algR* activates transcription of *algD* from the *algT*-dependent promoter [99]. The products of the genes *alg8*, *alg44* and *algK* are candidates for subunits of the alginate polymerase complex [88,89]. The products of *algX*, *algV*, *algI* and *algF* are periplasmic proteins involved in acetylation of polymeric mannuronic acid residues at the O-2 and/or O-3 positions [141] (Table 2). *A. vinelandii* *algJ* gene encodes an outer membrane protein (Table 2) whose derived amino acid sequence shared approximately 52% identity with product of *algE* from *P. aeruginosa* [109]. Further, the hydrophilicity profile as well as the amphipathicity of regions in the amino acid sequence showed significant similarities to that of *algE*. Based on these structural similarities and topological modeling, it is believed to be functionally equivalent, forming a pore involved in alginate export [109,111]. The alginate structural gene *algC* transcribes from two promoters, one of which is directly dependent on the alternative sigma factor [52]. In *A. vinelandii* seven unusual alginate biosynthetic genes identified as *algE1-7* encode a family of seven extracellular mannuronan C-5 epimerases (*AlgE1-7*) [35,54,132,133] (Table 2) which catalyze the Ca^{2+} -dependent polymer-level epimerization of β -D-mannuronic acid to α -L-guluronic acid (G) in alginate [34,132].

The *algE* epimerases consist of varying numbers of two types of structural modules, A and R, of which the A-module (one or two in each enzyme) alone is capable of catalyzing epimerization and determining the final distribution of G-moiety in the produced alginates [132,133]. The R-modules (one to seven copies) contain four to seven Ca^{2+} -binding motifs, stimulate epimerization rates and are probably involved in export of the *algE* proteins [2,36]. These extracellular Ca^{2+} -dependent epimerases generate a variety of epimerization patterns, including G blocks of various lengths. Such as *algE2* catalyzes the formation of G-blocks, while *algE4* introduces MG-blocks in polymeric mannuronans. *algE6* introduces contiguous stretches of G residues into its substrate (G blocks), while *algE7* acts as both an epimerase and a lyase [132]. The epimerase activity of *algE7* leads to formation of alginates with both single G residues and G blocks [54,132,133]. Along with *algE6* and *algE7* another alginate gene *algY* was also reported which have no epimerase activity, but a hybrid gene in which the 5'-terminal part was exchanged with the corresponding region in *algE4* expressed an active epimerase [132] (Table 2). These epimerase genes appear to be localized in three separate physical locations, in which *algE5* and *algY* are localized separately. Moreover, *algE7* is separated by approximately 5kb from the main block containing *algE1-4* and *algE6*. All of the enzymes therefore clearly do not originate from the same transcript. An operon organization in the block of six *algE* genes seems also unlikely, since the length of the region (25kb) and the spacing between the genes are larger than expected for an operon structure [132]. Surprisingly, the *A. vinelandii* chromosome also has an *algG* gene which encodes a Ca^{2+} -independent periplasmic mannuronan C-5-epimerase similar to the epimerase in *P. aeruginosa* [43,110] (Table 2), but its epimerization pattern has not been experimentally determined, due to low *in vitro* activity [54]. Sequence alignments demonstrate that *algG* does not belong to the *algE* gene family but shares 66% sequence identity to a mannuronan C-5-epimerase gene *algG* from *P. aeruginosa* [43]. In *A. vinelandii* and *P. aeruginosa* *algTmucABCD* gene products play similar regulatory roles in alginate biosynthesis since they are functionally interchangeable [84,100] (Table 2). In *A. vinelandii*, however, *mucB* that encodes a negative regulator of anti- σ^{22} factor which directly binds σ^{22} and target it for degradation [97,100]. *mucC* seems to function directly as a negative regulator of *algT* activity [8] but the mechanism of *algT* activity regulation remains to be determined. Loss-of-function mutations in either *mucA* or *mucB* have been reported to convert *P. aeruginosa* to mucoidy, by increasing *algT* activity [28,82]. In contrast, *A. vinelandii* strains produce alginate even in the absence of mutations in the *mucABCD* operon [101]. The *algR* gene from *A. vinelandii* has a high degree of homology with *P. aeruginosa* *algR* but the transcriptional regulation of the *A. vinelandii* *algR* gene and the role of *algR* in alginate production differ significantly from those of its *P. aeruginosa* counterparts [41,99]. These differences could be due to the fact that in *A. vinelandii*, alginate plays a role in encystment, a function not found in *P. aeruginosa* [99]. Inactivation of *A. vinelandii* *algR* diminished alginate production by 50%, but did not affect *algD* transcription, and completely impaired the capacity to form mature cysts. This implies that *algR* may exert some control over other alginate biosynthetic or regulatory genes and an

unidentified biosynthetic or regulatory alginate gene other than *algD* is under the control of *algU* [97]. Transcription of *algR* is not abrogated in the *algU* mutant strains; accordingly, the *algR* promoter does not have *algU* consensus sequences. This indicates the potential existence of another sigma factor involved in alginate and encystment control [99]. Whether phosphorylation of *A. vinelandii* *algR* is necessary for activation of its target cyst promoters remains to be investigated. Further studies will help to clarify the role of a response regulator such as *algR* in signal transduction and its interaction with *algU*-RNA polymerase.

Table 2. *A. inelandii* Alginate Genes, Known or Predicted Products and Functions

Gene	Gene product / function	References
<i>algD</i>	GDP-mannose dehydrogenase	[13,51]
<i>alg8</i>	subunit of alginate polymerase	[51,88]
<i>alg44</i>	subunit of alginate polymerase	[51,88]
<i>algK</i>	translocation/polymerization	[89]
<i>algJ</i>	porin-like OM protein	[109]
<i>algE1-7</i>	extracellular mannuronan C-5 epimerases	[35,54,132,133]
<i>algY</i>	hybrid of <i>algE4</i>	[132]
<i>algG</i>	mannuronan C-5 epimerases	[110,141]
<i>algX</i>	acetylation	[141]
<i>algL</i>	alginate lyase	[76,142]
<i>algV</i>	acetylation	[142]
<i>algI</i>	acetylation	[142]
<i>algF</i>	acetylation	[142]
<i>algA</i>	PMI-GMP	[77,141]
<i>algC</i>	PMM	[52,77]
<i>algR</i>	response regulator protein of TCSTS	[41,99]
<i>algT</i>	sigma factor σ^{22}	[84,97,100]
<i>mucA</i>	anti- σ^{22} factor	[84,100,101]
<i>mucB</i>	anti- σ^{22} factor	[51,84,100]
<i>mucC</i>	homologue of PhoORF4 product	[8,84,100,101]
<i>mucD</i>	homologue of serine protease (<i>HtrA</i>)	[51,84,100]

CONCLUSION

Alginate production in bacteria is under a complex regulatory control of a number of genes products, environmental stimuli and other factors. The genes involved in bacterial alginate production are found and can express only in rRNA homology group I *Pseudomonads* and *Azotobacter* representatives. In these bacteria, all alginate genes are chromosomal and most of them are transcriptionally organized into many operons. Much knowledge of alginate genetics has been

gained from *P. aeruginosa* than *A. vinelandii* and most of genetic aspects such as gene organization, function and transcription appear to similar but still some significant differences are there. The biosynthetic genes cluster in *P. aeruginosa* is under transcriptional control of *algD* except *algC* at 10-min whereas in *A. vinelandii* genes of biosynthetic cluster function as many transcriptional units. Further, *A. vinelandii* *algC* and *algD* have more than one functional promoters each behaves differently. Similarly in *A. vinelandii* epimerization steps are more complex and beside *algG*, seven different genes (*algE1-7*) products are involved than *P. aeruginosa*. In *P. aeruginosa* the transcriptional regulation of *algR*, *algB*, *algC* and *algD* is *algT* dependent whereas in *A. vinelandii* these genes transcribe independent of *algT* except one of promoters of each *algC* and *algD*. Although the most of genetic aspects of bacterial alginate have been extensively studied since last two decades and much knowledge has been gained. But still gaps are there especially the mechanism of polymerization and post polymerization modifications, is not well-understood. The ongoing research all over the world soon shall expectedly fill these unexplored gaps in near future.

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