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MICROBIAL PRODUCTION OF ALGINATE

Nadia Sharif[1], *Neelma Munir[1] and Shagufta Naz[1]

[1]*Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan*

*Corresponding author: neelma.munir@yahoo.com

ABSTRACT

Alginates are quite abundant in nature; they are hydrocolloids, water soluble biopolymers. Alginate is a natural polysaccride that occur as 30 to 60% structural component of brown algae. Alginate favorable properties like ease of gelation and biocompatibility promote its applications in engineering and bio medical science.

Keywords: Alginate review focus on the various aspects of microbial production of the present.

1. INTRODUCTION

Alginate occurs naturally as hydrophilic and anionic polysaccharide. It consequent mainly from bacteria and brown seaweed and is one of the most copious biosynthesized materials. Alginates were discovered in the late nineteenth era, are fundamental constituents of some microorganisms and brown algae of phaeophyceae group. They are alleged to numerous roles like capsular polysaccharides in soil bacteria *Azotobacter vindenadii*. Other than algae, the bacterial genera that produce alginates are Pseudomonas and Azotobacter (Johnson *et al.*, 1997; Skjåk-Bræk *et al.*, 1989; Narayanan *et al.*, 2012) .

It can be used as a food gelling agent, viscosifier, as a stabilizer and also have wide applications in paper, beverage, pharmaceutical and printing industries. About 30,000 metric tons alginate is formed yearly for industrial use from Macrocystis and Laminaria seaweed. In such seaweeds the alginate plays a structural role with 40% dry matter of the plant. This role of alginate is similar to terrestrial plants cellulose (Rowley *et al.*, 1999; Donati *et al.*, 2005; Hay *et al.*, 2013). Alginate has become a U.S. Food and Drug Administration (FDA)-approved polymer. It is significant biomaterials for varied solicitations in nutritional supplements, regeneration medicine and also in semipermeable separation. For three-dimensional scaffolding materials it is readily process able for applicable like microcapsules, hydrogels, sponges, foams, fibers and microspheres (Rowley *et al.*, 1999 ; Bouhadir *et al.*, 2001).

1.1 Alginate: Characteristics

Commercially presented alginate is usually derived from Phaeophyceae group of algae, comprising *Ascophyllum nodosum*, *Macrocystis pyrifera*, *Laminaria digitata*, *Laminaria hyperborean* and *Laminaria japonica*. Extraction is made by the aqueous alkali solution ssuch NaOH use. After filtration the alginate is precipitated by adding calcium or sodium chloride. Alginic acid is produced from alginate salt through dilute HCl treatment. Subsequently water-soluble sodium alginate power is produced through purification and conversion steps. About 25–44% for *Laminaria digitate* and 22–30% for *Ascophyllum nodosum* are alginate contents on dry weight basis (Rinaudo, 2008). A more physical properties and chemical structured alginate is obtained from bacteria rather than seaweed.

Pseudomonas and Azotobacter are the main sources of bacterial alginates. The biosynthesis pathway of Alginate is usually divided into (i) cytoplasmic membrane transfer along with polymerization, (ii) modifications after periplasmic transfer (iii)

precursor substrate synthesis, and (iv) transfer to outer membrane. Current advancement in controlling the synthesis of alginate in bacteria, and the comparative affluence of bacterial alteration might facilitate the making of alginate with tailor-made characteristics and varied solicitations in biomedicine uses (Qin, 2008).

1.2 Structure and Characterization

Alginate is a family of unbranched binary co-polymers of α-L-guluronic acid (G) and α (1–4) linked β-D-mannuronic acid (M) of generally erratic configuration and sequential structure. Azotobacter alginates are true block co-polymers, composed of homopolymeric regions of M and G blocks, interspaced with regions of alternating structure (MG blocks). The major component of alginate was considered to D-mannuronate till Fischer and Dörfel recognized the L-guluronate residue. It have been confirmed through partial precipitation with the salts of calcium and manganese that Alginates are block copolymers, the proportion of mannuronate and guluronate depends on natural source. Currently Alginate is recognized as a whole family of linear copolymers that contains α-L-guluronate (G) blocks of (1,4)-linked and β- D-mannuronate (M) residues. The blocks are consisting of successive G residues (GGGGGG), uninterrupted M residues (MMMMMM), and discontinuous M and G residues (GMGMGM). More than 200 different alginates are presently produced. Alginates take out from diverse sources diverge in the length of each block as well as M and G contents. The commercially available alginates have 14.0–31.0 G-block content whereas the Laminaria hyperborean stems have 60% (Qin, 2008). Just G-blocks of alginate are alleged to contribute in intermolecular cross-linking with divalent cations (e.g., Ca^{2+}) to make hydrogels. The configuration (i.e., M/G ratio), G-block length, molecular weight and sequence are therefore precarious features that affect the alginate physical characteristics and its subsequent hydrogels. By increasing the molecular weight and the length of G-block the mechanical assets of alginate gels generally are enhanced. It is imperative that the altered alginate cradles give polymers with a range of chemical structures (e.g., bacterial alginate made from Azotobacter has a high concentration of G blocks and its gels have a comparatively elevated arduousness. The stability of the gels is dependent upon the rate at which drugs is released from the gel, physical properties of the alginates, function and phenotype of alginate gel encapsulated cells (George and Abraham, 2006; Hay *et al.*, 2010).(Figure 1)

1.3 Genetics of alginate biosynthesis

Though the regulation is different but the *Pseudomonas* and *Azotobacter* genes that are involved in biosynthesis of alginate are fundamentally alike. Entire nonetheless as one core genes convoluted in the synthesis of alginate are confined in a single 12-gene operon originally defined algD, alg8, alg44, algK, algE (algJ), algG, algX, algL, algI, algJ (algV), algF and algA. In parentheses the Pseudomonas gene names are with the conforming Azotobacter gene names (Chitnis and Ohman, 1993). Promoter upstream of algD controls the genes expression. Within the operon, there is also substantiation for the alternate internal promoter's existence. The gene algC is also involved in lipopolysaccharide and rhamno-lipid synthesis while this gene is not located within the operon. There are additional to these 13 core genes that are convoluted in the synthesis of alginate.

1.4 Microbial biosynthesis of alginate

Bacterial genera Azotobacter and Pseudomonas secrete alginate. The soil dwelling *Azotobacter vinelandii* and unscrupulous human pathogen *Pseudomonas aeruginosa* have been used extensively to uncover the molecular mechanisms involved in biosynthesis of bacterial alginate. Though the mechanism by which these bacteria produce alginate in nature is analogous but the secreted alginates possesses different material properties as well as they are secreted by different purposes. The development of thick highly structured biofilms are aided by some mucoid strains such as *P. aeruginosa* discharge ample quantities of alginate (Hay *et al.*, 2009; Nivens *et al.*, 2001). With higher concentrations of G residues, *Azotobacter* produces a stiffer alginate that permits the development of dehydration resilient cysts that rests diligently concomitant with the cell (Sabra and Zeng, 2009). There are four steps for the biosynthesis of alginate: precursor synthesis, polymerization, periplasmic modification/transit and transport.(Table1)

1.5 Synthesis of precursor

The formation of the activated precursor guanosine diphosphate (GDP)-mannuronic acid is a defined process. It includes a succession of cytosolic enzymatic phases that feed in the membrane bound alginate polymerization machinery (Figure 2 and 3).

To start the biosynthesis, six carbon substrates enter into the KDPG pathway; it results in to pyruvate synthesis, which is further channeled headed for the tricarboxylic acid cycle. Consequently, fructose-6-phosphate is produced from oxaloacetate through gluconeogenesis (Lynn and Sokatch, 1984; Narbad *et al.*, 1987). Four next biosynthesis steps are carried out through AlgA, AlgC and AlgD defined enzymes, in which GDP mannuronic acid is produced from fructose-6-phosphate.

Firstly, the mannose- 6-phosphate is produced from fructose-6-phosphate by phosphomannose isomerase action of the bifunctional protein AlgA. At that point, mannose-1-phosphate is produced from mannose-6-phosphate by the action of AlgC phosphomannomutase (May *et al.*, 1994; Zielinski *et al.*, 1991). It resulted in to GDP mannose formation that is catalyzed by the GDP-mannose pyrophorylase action of AlgA through GTP hydrolysis. Remarkably, the AlgA catalyzed step proceed the reverse reaction, nevertheless the yank of the consequent AlgD proceeded step moves the reaction towards GDP-mannose manufacture.

GDP-mannose dehydrogenase (AlgD) catalyzed the irreversible final step and results in GDP-mannuronic acid, that acts as the precursor for the polymerization technology of alginate. The alginate synthesis pathway, the oxidation step that is catalysed by the AlgD is a key rate-limiting reaction (Shinabarger *et al.*, 1991; Tatnell *et al.*, 1994).

1.6 Polymerization

In the process of the alginate biosynthesis the polymerization and translocation are comparatively indisposed assumed progressions. It is shown from the disruption mutagenesis that no less than two proteins are prerequisite for polymerization: the inner membrane (IM) proteins Alg44 and Alg8. If the alginate synthesizing genes are disrupted then it results either in one of three phenotypes. It may resulted in no alginate fragments production or complete alginate production loss as for Alg8 and Alg44. It may result in production of alginate, as in the case of AlgI, AlgJ and AlgF. It can also resulted in alginate production loss nevertheless the discharge of

alginate fragments because of periplasmic alginate lyase action, as for AlgX, AlgG, AlgK and AlgE (Franklin and Ohman, 2002; Jain and Ohman, 1998; Remminghorst and Rehm, 2006).

Through Bioinformatic analysis the best analyzed candidate for a polymerase is Alg8. It is prophesied that the transference of a sugar molecule from an activated donor to an acceptor molecule (e.g. a growing carbohydrate chain) is preceded through a glycosyltransferase (family-2 GT) action. In accord through functional analogous transmembrane glycosyltransferases for example cellulose synthase (AcsAB) and chitin synthase (Ch1), Alg8 displayed a anticipated structure with numerous transmembrane domains flanking a extended cytoplasmic loop compliant conserved motifs and catalytic filtrates.

The involvement of Alg8 in alginate polymerization has also been perceived after further experimentation. a supermucoid phenotype is recovered due to an overexpression of Alg8 headed to the overproduction of alginate.

The overproduction would appear to propose that in the biosynthesis pathway; bottleneck is the Alg8 catalyzed polymerization reaction. Stimulatingly, it is shown from the in-vitro polymerization investigation that the whole cell envelope (IM and outer membrane plus associated proteins) was essential for polymerization, it suggests that Alg8 need other proteins for their action (Hay *et al.*, 2009; Remminghorst *et al.*, 2009).

Alg44 is considered to act an indirect role whereas the exact mechanism and the specific role that Alg44 plays in polymerization is still need to be explored. As for the alg8, there will be no alginate polymerization by the deletion of alg44 gene. On the other hand its overexpression directed to the overproduction of alginate. Alg44 is anticipated as a multidomain protein consisting of a transmembrane region, a cytoplasmic PilZ domain and a periplasmic domain that displays homology to the membrane fusion protein MexA, a membrane-bridging protein convoluted in the multidrug efflux system of *P. aeruginosa*. It is proposed from the periplasmic membrane fusion protein domain that Alg44 may display a physical role in linking the membrane bound polymerase to the periplasmic and outer membrane constituents enabling the transit, alteration and discharge of alginate (Oglesby *et al.*, 2008; Remminghorst and Rehm, 2006). The Alg44 cytosolic PilZ domain bind to the bacterial secondary messenger bis-(3′–5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) and is necessary for the synthesis of alginate (Merighi *et al.*, 2007).

1.7 Periplasmic transfer and modification

In the biosynthesis process, the preeminent studied proteins are the Alginate altering enzymes and indulgent of the reaction configuration of such enzymes will permit the production of tailor made alginates. Material assets are dependent chiefly on composition and structure of the alginate, so water-binding, immunogenic and gel-forming properties of the polymer are depends on the comparative volume and sequence dissemination of guluronic acid and mannuronic filtrates (Ertesvåg *et al.*, 1999). Modification of bacterial alginates completely ensues in the periplasm that suggests, the alginate is produced as polymannuronate and the aforementioned alteration happens at polymer phase. Moreover, *A. vinelandii* genome codes a family of seven extracellular Ca2+- dependend epimerases (AlgE1-7) and the genes that encode these extracellular epimerases are not placed in the cluster

of alginate synthesizing gene. Currently In *Laminaria digitata* genome, six diverse C5-epimerase encoding genes have been known (Bjerkan *et al.*, 2004; Ertesvåg *et al.*, 1999; Nyvall *et al.*, 2003; Valla *et al.*, 2001). It has been suggested from primroses large gene family, nevertheless alike biological roles they perform, there enzymes perform numerous functions. The genes that are directly convoluted in nascent alginate chain alteration are located in operon of bacterial biosynthesis, it includes, alginate lyase, AlgI, AlgJ, AlgF like acetylation-complex encoding genes and the mannuronan C5-epimerase AlgG and AlgL. In the last few years the genes have been intensively characterized and cloned (Bakkevig *et al.*, 2005; Chitnis and Ohman, 1990; Franklin and Ohman, 1993; Gimmestad *et al.*, 2003; Shinabarger *et al.*, 1993). At hydroxyl-groups and C2 and/or C3 position of the mannuronic acid deposit, the transacetylation avert such residues from being epimerized to guluronic acid and after degradation by the alginate lyase (Franklin and Ohman, 1993).

Consequently, indirectly the periplasmic acetylation regulates the degradation and epimerization of the alginate polymer. As acetylation degree is increased in order to survive under the dehydrating conditions the water binding aptitude of alginate is stalwartly improved (Nivens *et al.*, 2001). For alginate biosynthesis, the acetylation itself is not needed, though the algI, algJ and algF genes are prerequisite to add the O-acetyl groups to the alginate polymer. Subsequently the polymer epimerization remained significant in acetylation-negative mutants of *P. aeruginosa*, in the periplasm the acetylation complex AlgIJF is not part of the alginate defending and aligning scaffold (Franklin and Ohman, 2002).

The AlgF and AlgJ proteins are primarily traced in the periplasm, whereas AlgJ is similarly fastened in the cytoplasmic membrane probably by the signal peptide hydrophobic part. AlgI provides the unidentified cytosolic substrate for acetylation, traverse the cytoplasmic membrane and is a 7-helical transmembrane protein. According to the epimerization of alginate, the *Azotobacter* and *Pseudomonas* varies. Mostly, the transformation of unacetylated b-D-mannuronic acid residues is catalyzed by the epimerases in to C-5-epimers a-L-guluronic acid. Though a periplasmic mannuronan C5-epimerase (AlgG) is present in both *Azotobacter* and *Pseudomonas* yet the extracellular epimerases in Azotobacter dditionally alters alginate by AlgE1-7 (Ertesvåg *et al.*, 1999; Rehm *et al.*, 1996). With its periplasmic counterpart on amino acid sequence level there is no similarity in extracellular epimerases. Intriguingly, each of these seven catalysts presented *in vitro* an alternate trademark succession conveyance of G-deposits in the polymannuronate substrate, which may clarify the broad inconstancy in the alginate created by *A. vinelandii* (Ertesvåg *et al.*, 1999). Such broad fluctuation may be organically noteworthy for the arrangement of the residing-organize, assigned blister,that is trademark for the family Azotobacter (Campos *et al.*, 1996; Høidal *et al.*, 2000). Additional biotechnological potential have been confirmed through the hybrid epimerase expression in *E. coli*, because the as compared to their parent enzymes, the epimerization configuration of the hybrid enzymes were dissimilar (Bjerkan *et al.* 2004).

The guluronic corrosive substance of the polymer compares to more communication regions with divalent cations (particularly Ca2+), bringing about additional unbending and gel-like polymer

configurations. Therefore, shuffling or genetic engineering of epimerases strappingly improves the alginate design space (Bjerkan *et al.*, 2004). The lyases of alginate that are also recognized as alginate depolymerases or alginases is involved in catalyzing the b-elimination reaction that ultimately lead to the squalor of alginate. Usually, all lyases precede a similar response, yet corresponding the epimerases, every unique lyase is characterized by its substrate related characteristics and breakage design. Certain lyases are inclinations for glycosidic securities amid mannuronate deposits, though others just utilize polyguluronate as substrate. In alginate creating microscopic organisms, the lyase apparently works as an altering compound regulating the polymer atomic weight and length. Alginate delivering microbes can't reuse the incorporated alginate (Boyd *et al.*, 1993). Additionally proteins are intricate in the scaffold development that defends and make parallel the promising alginate chain even though navigating the periplasm. The algX and algK deletion occasioned in the free uronic acids discharge that is because of degradation of alginate lyase (Jain and Ohman, 1998). The periplasmic serine protease/chaperone like protein MucD is accounted for effect on control of alginate creation. Inactivity of the projected protease MucD or cancellation of its quality brought about unchecked biosynthesis of alginate in *P. aeruginosa*, phenotypically demonstrated by alginate creation and expanded temperature affectability. Presentation of plasmid encoded mucD in these mutants reestablished control of alginate biosynthesis, i.e. reestablishing the first non-alginate delivering phenotype (Jain and Ohman, 1998).

1.8 Alginate export through the outer membrane

Firstly it was described in 1990 that the outer membrane beta barrel porin, AlgE, is accountable for the discharge of mature alginate (Grabert *et al.*, 1990; Rehm *et al.*, 1994b). Described protein is immunogenic and shows anion discernment on unprompted integration into planar lipid bilayers. As of late, the crystal structure of AlgE was resolved and utilitarian buildups of protein completely examined. Electrophysiological and biochemical eexaminations of AlgE recommended a capacity in alginate export. In topological reviews Rehm and associates found that AlgE framed an anion particular pore via external layer, andlipid bilayer tests demonstrated that the pore may incompletely hindered by GDP-mannuronic corrosive (Rehm *et al.*, 1994 a). The protein AlgJ in *A. vinelandii* parts great likeness with AlgE, and is talked about to apply an indistinguishable capacities from its *Pseudomona* partner (Rehm *et al.*, 1996).

1.9 Regulation

The control of alginate synthesis is perplexing and includes transcriptional and post-translational levels of direction, and also a few hyper mutable areas of the genome in which mutant alleles prompt alginate overproduction. All inclusive acting controllers and a few alginate-particular controllers oversee synthesis of alginate. Alginate synthesis, transcriptional control in *P. aeruginosa* could be loosely isolated into two distinct sorts: natural jolts based control and a 'genotypic switch'- grounded type of direction. Mostly genes that are convoluted in the genotypic shift are positioned inside a solitary operon that is self-operative (algU, mucA, mucB, mucC and mucD). In *E- coli* this area is somehow similar to the well categorized σE region, contains the genes rpoE (encoding the σE), rseA, rseB, rseC. AlgU is a significant substitute σ22 factor, that is at the peak of regulators hierarchy and convoluted in biosynthesis

of alginate and is eventually needed for transcription preparatory from the promoter AlgD (Chitnis and Ohman, 1993). AlgU is confiscated by the side of the IM by the membrane anchored antisigma factor MucA, consequently incapable binding to RNA polymerase and inductee transcription (Xie *et al.*, 1996). MucB periplasmic protein impasses to the MucA periplasmic side, in the biosynthesis of alginate it displays a adverse control through MucA protection from proteolysis.

Release of AlgU and consequent translation has all the earmarks of being started through a regulated intramembrane proteolytic (RIP) course prompting the dilapidation OF MucA. A few stages of the RIP cascade have as of late become known: MucA is cut down in the light of envelope stress through the AlgW (*E. coli* DegS homologue) periplasmic protease initially. Specific misfolded proteins (specifically, the external film protein MucE) can tie to the PDZ actuating area of AlgW and make its initiation (de-restraint) possible. Subsequently AlgW, MucA cleavage winds up noticeably helpless to cleavage on the cytosolic side by the intramembrane protease MucP (E. coli RseP/YaeL homologue) prompting the arrival of AlgU. ClpX, ClpP1 and ClpP2 cytosolic proteases have as of late been appeared to be required in the proteolysis of MucA (Qiu *et al.*, 2008).

Periplasmic protease MucD gives off an impression of being assuming a part adversarial to AlgW. Disturbance of mucD quality prompts a mucoid phenotype implying a adverse administrative part. It is believed that MucD is comprised in the corruption of misfolded proteins that will somehow or another initiate AlgW or MucP (Qiu *et al*., 2008). Despite the fact that alginate generation is the greatest evident phenotype regulated by AlgU, it doesn't turn solely upon alginate operon and appeared to be required in the transcriptional actuation of qualities through differing capacities, incorporating qualities required in biosynthesis of different exopolysaccharides (Ghafoor *et al.*, 2011).

This operon has turned out to be recognized as the "switch" locus in view of the generally large number of transformations set up in such area in clinical mucoid disengages. Widely recognized transformations happen in MucA as well as MucB, by 80% of *mucoid P. aeruginosa* clinical confines encompassing changes in the mucA quality. A large portion of these transformations result in an untimely stop codon and a truncated MucA rendering the RIP course repetitive. Notwithstanding AlgU, a few different proteins are prerequisite to start translation of the alginate operon. This directional sequence is recognized as 'ecological jolts'- grounded control. AlgR is a reaction controller portion of a two segment controller that ties to three locales in the algD promoter; the related tactile segment of this administrative combine is AlgZ (FimS) and abnormally is not obligatory for translation of the alginate operon. AlgB is additionally part of a two-segment controller and ties to one site on the algD promoter; once more, it's movement is evidently free of its related sensor kinase KinB. AmrZ (initially called AlgZ), an Arc-like DNA-restricting protein, ties to one site on the algD promoter (May *et al.*, 1994; Qiu *et al.*, 2008).

1.10 Applications of Alginate

All marketable alginates are confined from cultivated brown seaweeds, with more than 30,000 metric tons delivered yearly. The flexibility, biocompatibility and material properties has prompted alginates use as a stabilizer and consistency controller in sustenances,

beauty care products and high-esteem therapeutic applications including wound dressings, sedate conveyance frameworks and all the more as of late in tissue exemplification for regenerative treatment (Qin, 2008; Tønnesen and Karlsen, 2002). The most broadly analyzed utilization of alginate gels to advance vein development has misused their capacity to give a managed and limited arrival of heparin restricting development elements, for example, vascular endothelial development consider (VEGF) (Lee and Mooney, 2012).

Sodium alginate could be utilized as an authoritative and deteriorating agent in tablets, as a stabilizer for emulsions and as a appending and congealing operator in creams, water-miscible gels and salves. The potential falsehoods, in any case, in the advancement of alginate-controlled medication conveyance frameworks. Generally, sodium alginate are utilized as a tablet restricting agent, whereas alginic corrosive is utilized as a tablet disintegrant in compacted tablets intended for quick medication discharge (Tønnesen and Karlsen, 2002).

Alginate gels are broadly inspected in the course of recent decades as a vehicle to convey cell populaces or proteins which coordinate the recovery or designing of different organs and tissues in the body. The different uses of alginate gels are subjugated the extensive variety of gelling methodologies, physical assets, cell attachment, and debasement conduct of this group of constituents (Lee and Mooney, 2012).

Alginate has a few characteristics that are empowered it to be utilized as a lattice for the entanglement or conveyance of cells, DNA and protein like biomolecules. Raft-forming formulations of alginate are widely applied in the management of esophagitis and acid reflux (Tønnesen and Karlsen, 2002). In biomedical reviews, for mammalian cell culture model, the alginate gels are progressively being used as a classical framework. These gels can be promptly adjusted to fill in as either 2-D or all the physiological significant 3-D culture frameworks. Alginate gels have benefit for bone and ligament recovery, when contrasted with different constituents, because of their capacity to be brought into the body in a negligibly intrusive way, controlled arrival of tissue acceptance components, the simplicity of compound adjustment with grip ligands and their capacity to fill unpredictably formed deformities. Alginate gels are additionally being effectively examined for their capacity to intervene the recovery and designing of an assortment of different muscles and organs, like liver, pancreas, skeletal muscle and nerve (Lee and Mooney, 2012).

Hepatic tissues replacement in case of liver failure is now possible through tissue engineering and hepatocytes can be encapsulated in alginate gels. Hence the alginate provides appropriate platform for the development of a bio artificial liver for instance they are easy to cryopreserve and manipulate (Lee and Mooney, 2012).

CONCLUSION AND FUTURE PERSPECTIVES

Alginate is currently entrenched in numerous industries significantly playing an important role as a dressing material of wounds in clinical use. Alginate is an important compound pharmacologically and for treatment of type 1 diabetes, it includes islet transplantation. However, alginate gels have very inadequate mechanical stiffness and general physical characteristics as compared to other hydrogels. Matching the specific application with physical properties of the alginate is the biggest task. In near

future, the alginate-based materials use is foreseen, whereas presently their fairly passive role is their use in wound healing. Forthcoming vinaigrettes drive a copious role.

Wound healing bioactive mediators may be assimilated into alginate vinaigrettes, because such gels have proved their effectiveness in sustaining confined effective biological elements, like proteins, for prolonged time periods. For drug delivery particularly in addition to wound healing commonly, a particular requirement in response to environment variations is the control of sequential and sustained as well as multiple and single drug response.

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Table 1: Proteins involved in alginate biosynthesis

Protein	Description	Subcellular location	Reference
AlgA	Precursor synthesis. Phosphomannose isomerase/GDP-mannose pyrophosphorylase.	Cytosol	(Shinabarger *et al.*, 1991b)
AlgC	Precursor synthesis. Phosphomannomutase. PDB: 1P5G	Cytosol	(Ye *et al.*, 1994)
AlgD	Precursor synthesis. GDP-mannose dehydrogenase. PDB: 1 MV8	Cytosol	(Tatnell *et al.*, 1994)
Alg8	Polymerization. Proposed glycosyltransferase/polymerase.	IM	(Remminghorst *et al.*, 2009)
Alg44	Polymerization and post transcriptional regulation. c-di-GMP binding and response.	IM	(Remminghorst and Rehm, 2006a)
AlgK	Export/structural role. Lipoprotein, Stabilizes AlgE in OM. PDB: 3EB4	Associated with periplasmic side of OM	(Keiski *et al.*, 2010)
AlgE	Export. OM porin. Named AlgJ in *Azotobacter*PDB: 3RBH	OM	(Whitney *et al.*, 2011)

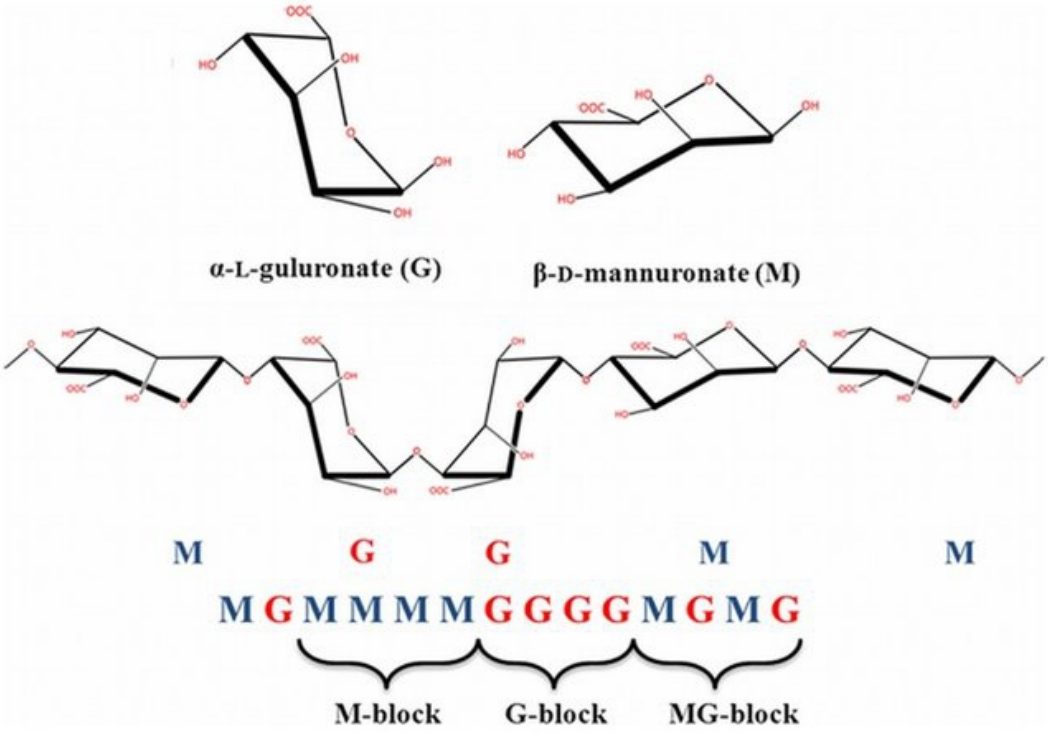

Figure 1: Structure of alginate
(Source: Paredes-Juárez *et al*., 2016)

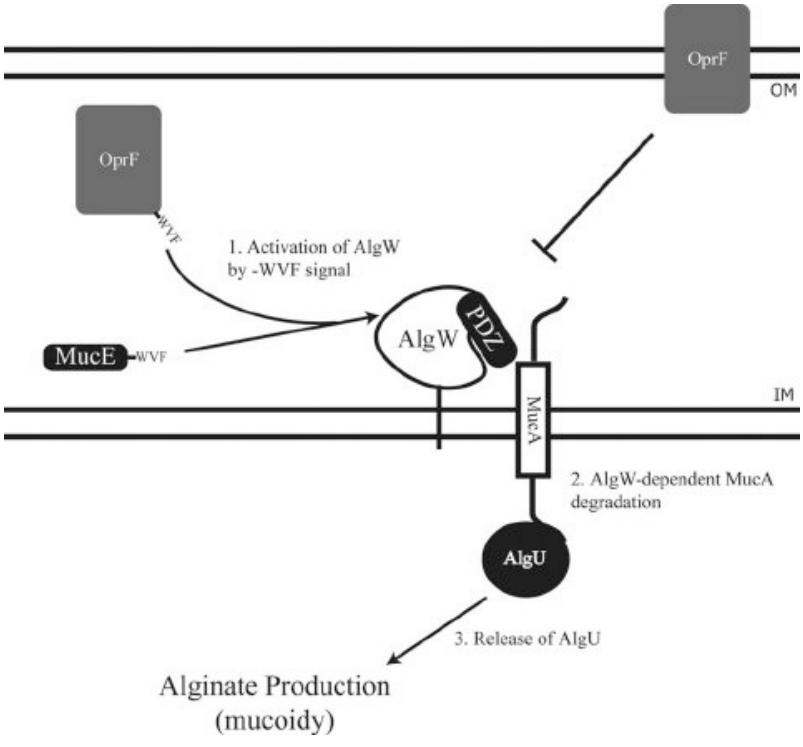

Figure 2: Regulation of alginate production
(Source: Qiu *et al*., 2008)

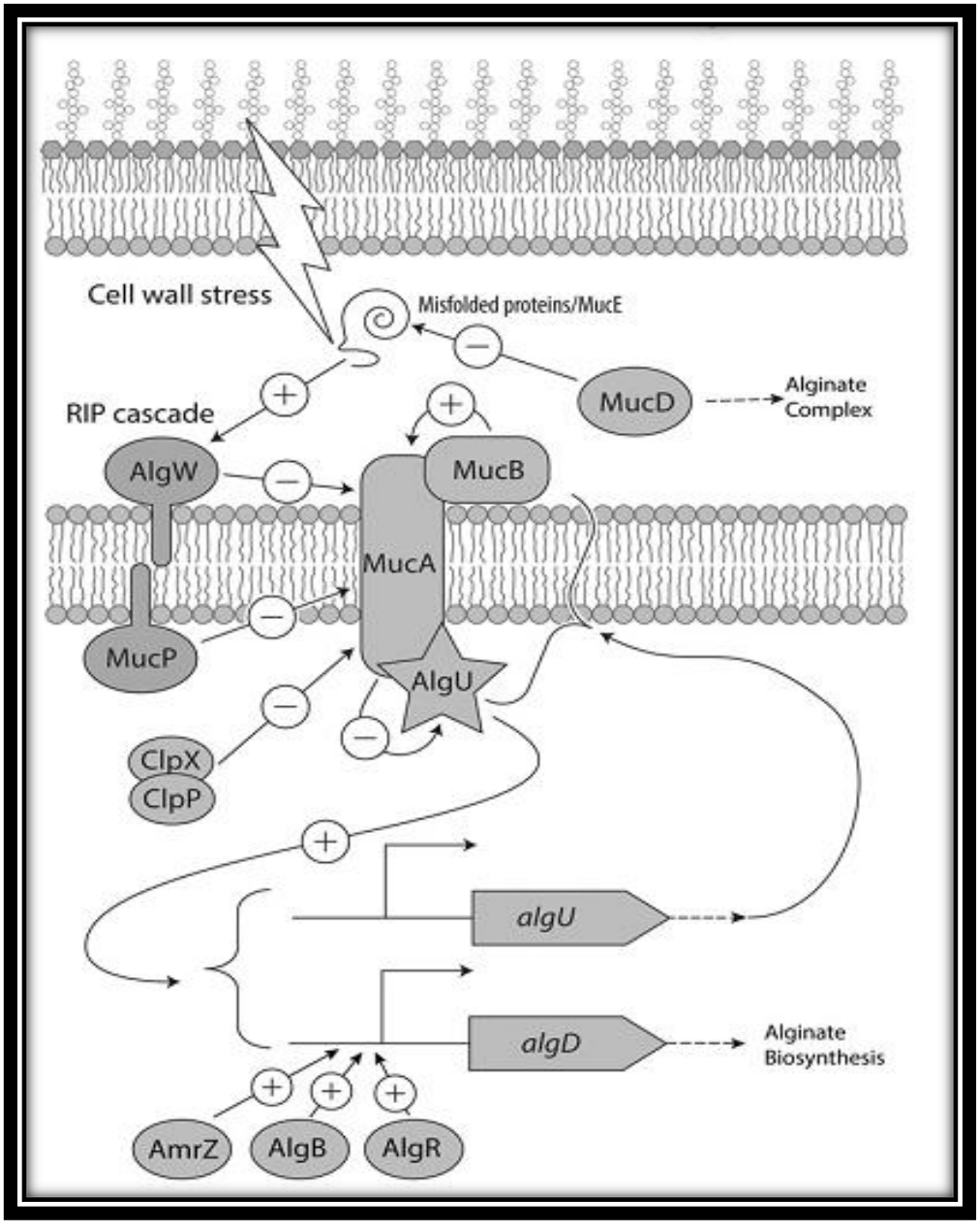

Figure 3: Overview of regulation of alginate biosynthesis (Source: Hay *et al*., 2013)