Cloning of Alginate Lyase Gene (alxM) and Expression in *Escherichia coli*†

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The *alxM* gene encoding a d-mannuronan-specific alginate lyase has been cloned from a marine bacterium isolated as an epiphyte on the brown alga, *Sargassum fluitans*. Expression of this gene in *Escherichia coli* provides a source of this enzyme for probing alginate structure and modifying the mannuronan-rich alginate polymers produced by bacterial pathogens.

Alginate is an acidic polysaccharide comprised of 1→4 linked β-D-mannuronic acid and α-L-guluronic acid residues. It is the predominant structural component of brown algae (1) and is secreted as an extracellular polysaccharide by species of *Azotobacter* and *Pseudomonas* (11, 14, 16). The secretion of mannuronan-rich alginate by mucoid strains of *Pseudomonas aeruginosa* has been correlated with their relative virulence as opportunistic pathogens associated with cystic fibrosis (17). Similarly, secretion of alginate with mannuronic acid residues as the predominant if not exclusive component has been implicated in the phytopathogenic properties of several pathogens of *Pseudomonas syringae* (12).

Alginate lyases (EC 4.2.2.3) cleave the 1→4 glycosidic linkage of the uronic acid polymer by a β-elimination reaction resulting in an unsaturated nonreducing terminus. These enzymes have been detected in a variety of microorganisms (2, 9, 10, 15, 24), and known enzymes have a preference for either L-guluronic or D-mannuronic acid residues. The guluronic-specific alginate lyase of *Klebsiella pneumoniae* was cloned and expressed in *Escherichia coli* (5). The extracellular L-guluronic-specific alginate lyase from *Klebsiella aerogenes* type 25 has been used to investigate alginate structure (3, 6, 8). However, a mannuronan-specific enzyme is not readily available for complementary investigations.

Bacteria associated with pelagic species of the brown algal genus *Sargassum* secrete alginate lyases which have a strong preference for either the D-mannuronan or L-guluronic components of alginate (18, 19, 21, 22). One of the facultative isolates, SFFB080483Alg-A (ATCC 433367), secreted an endolytic, mannuronan-specific lyase which was homologous on the basis of biochemical studies (21). This gram-negative bacterium was selected for construction of a gene library. Here, we report the cloning of the *alxM* gene encoding the mannuronan-specific lyase and its expression in *E. coli*.

The gram-negative marine isolate, SFFB080483Alg-A (19), was grown as previously described in Provao’s enriched seawater medium containing 0.1% alginate (18). High-molecular-weight DNA was prepared by a combination of enzymatic digestion and cesium chloride centrifugation (4). Chromosomal DNA was partially digested with *Sau3A* and 4- to 6-kb fragments were purified by agarose gel electrophoresis. This DNA was ligated into the dephosphorylated *Bam*HI site of pUC18 (7). Transformants were selected in *E. coli* TC4 on Luria agar containing 50 µg of ampicillin per ml. A random sample of these indicated that more than 90% contained 4- to 6-kb DNA inserts.

Approximately 2,400 clones were screened for alginate lyase activity. Colony lifts were made by using Whatman no. 1 filter paper circles cut to fit inside a petri plate. After exposure of the colonies to chloroform vapors for 5 min, the cell side of the filter was placed on the surface of petri plates containing 1% agarose and 0.1% alginate in buffer (30 mM potassium phosphate, 50 mM KCl, pH 7.5). Preparations were incubated overnight at 37°C. Filters were removed and the plates were stained by flooding with 0.05% ruthenium red (20). Clear zones of depolymerization on a red background were observed, indicating lyase activity. Twelve lys-positive clones were isolated during the screening process. No zones of depolymerization were observed around control strains containing pUC18. The most active clone, pAL-A3, produced a 25-mm zone of clearing and was investigated further.

A restriction map was constructed for pAL-A3 as shown in Fig. 1. The plasmid contains a 4.1-kb genomic DNA insert. The origin of the *alxM*-containing fragment of DNA was confirmed by Southern hybridization to chromosomal DNA from SFFB080483Alg-A (Fig. 2) by using a 1.6-kb KpnI fragment of pAL-A3 as a probe (23). The pattern of hybridization to chromosomal digests was consistent with the presence of a single chromosomal copy of this gene in SFFB080483Alg-A.

The alginate lyase from strain SFFB080483Alg-A has been reported to be specific for d-mannuronan (22), and this specificity was examined in the recombinant *E. coli* TC4(pAL-A3). Cells were grown to late exponential phase and treated to release periplasmic enzymes (25). The yield of enzyme from the transformed *E. coli* periplasmic preparation is 1.70 units per ml of culture broth, compared with 0.40 units per ml of culture broth from the parent organism (21). A432 was monitored to detect the unsaturated nonreducing terminus in the lyase-generated product. Homopolymeric substrates were prepared by mild acid hydrolysis of alginate (13). Reactions were run in 0.43 M sodium phosphate buffer (pH 7.5) containing 0.5 M NaCl and 0.02% uronic acid polymer (Fig. 3). The rate of product formation was higher with D-mannuronan than with mixed polymers of alginate.

L-Guluronic was not degraded by the enzyme released from *E. coli* TC4(pAL-A3). Alginate lyase with L-guluronic spec-

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further evidence that plasmid pAL-A3 is identical to that reported previously for the dominant lyase present in SFFB080483Alg-A (20), providing further evidence that the plasmid contains the alxM gene.

The cloning and expression in E. coli of a gene coding for the D-mannuronan-specific alginate lyase will provide an abundant source of this enzyme to examine the relationships between enzyme structure, substrate specificity, mechanism, and regulation. The D-mannuronan-specific enzyme also provides a complement to the L-guluronan-specific alginate lyase from K. aerogenes type 25, which has been used to probe the structural organization of alginate from brown algae (3), Azotobacter vinelandii (8), and pathogenic P. aeruginosa (6). The availability of the alxM gene product should be of particular value because of its specificity for D-mannuronan, which is the major component of alginate secreted by Pseudomonas sp. plant (12, 16) and animal pathogens (11, 17).

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REFERENCES


