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Selective alginate degradation by marine bacteria associated with the algal genus *Sargassum*

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SUMMARY

Alginase-secreting bacteria associated with actively growing tissues of the marine Phaeophyta species *Sargassum fluitans* and *S. natans* have been isolated and evaluated for their ability to degrade alginate (ALG), carboxymethylcellulose, and agar. Of seven isolates selected for their ability to grow on 2% agar containing 1% sodium alginate, none were able to grow on either 2% agar or 2% agar supplemented with 0.1% carboxymethylcellulose. Two of these with fermentative potential, i.e., ALG-A and ALG-G, showed selective activities with respect to their ability to degrade native alginate and/or take up the products resulting from alginate degradation. The ALG-A isolate was able to rapidly degrade native alginate with the generation of a stable polymer fraction and small oligouronides, most of which were dissimilated for growth. The ALG-G isolate was able to completely degrade native alginate with the accumulation of significant quantities of unsaturated dimeric and trimeric oligouronides. A limit polymer was generated from the action of a polymannuronan-specific extracellular alginate lyase purified from exponential cultures of the ALG-A organism. This product proved to be an effective substrate for the alginate lyase activity obtained from the medium of exponential phase cultures of the ALG-G isolate, and upon incubation with concentrated and dialyzed ALG-G medium was converted to the products that were observed to accumulate in the medium of the ALG-G isolate grown on native alginate. These organisms represent examples of the microflora associated with actively growing *Sargassum* tissues, each with a selective ability to degrade and dissimilate the biomass of the marine brown algae.

INTRODUCTION

The enzyme-mediated degradation of alginate has been shown to occur predominantly if not exclusively through transeliminase reactions in which the cleavage between the C-4 and the glycosidic oxygen of the 1–4 glycosidic bonds is accompanied by the introduction of unsaturated bonds between carbon atoms 4 and 5 of the D-mannuronate (ManA) and L-guluronate (GulA) moieties of the alginate polymer. Enzymes catalyzing these reactions have been designated as alginate lyases and have been found in a number of invertebrate animal and bacterial species [2–5,7–13,15–17]. With objec-

tives of identifying bacteria associated with the marine brown algae that participate in the degradation of the biomass and obtaining alginate lyases with different substrate specificities, we have isolated several bacteria from actively growing tissues of the pelagic *Sargassum* species, *S. fluitans* and *S. natans*, and have investigated the properties of the intracellular and extracellular alginate lyases produced by oxidative and fermentative isolates [13,15,16]. In this communication, we have examined the potential of several of the alginase-secreting isolates to degrade carboxymethylcellulose and agar. Two of the fermentative isolates have been evaluated for their ability to selectively degrade and dissimilate alginate in relation to their ability to secrete alginate lyases specific for poly(ManA) and poly(GulA) components of the alginate polymer.

MATERIALS AND METHODS

Chemicals and reagents

Alginate (ALG) was obtained from Fisher Scientific Company as a purified grade isolated from species of *Macrocystis*. Carboxymethylcellulose (CMC) was obtained as the sodium salt of the high viscosity grade (1300–2200 centipoises for a 1% aqueous solution at 25°C) from Sigma Chemical Company. Agar was Bacto-Agar® from Difco. Tetrabutylammonium hydroxide was from Sigma. Acetonitrile was HPLC grade from Fisher. All other chemicals were reagent grade. Deionized water was used for the preparation of media; deionized and glass-distilled water was used for the preparation of all other reagents.

Isolation and growth of bacterial cultures

Alginate-secreting bacteria were dislodged from actively growing tissues of *Sargassum* by mild sonication in sterile artificial sea water and isolated on the basis of the appearance of clearing zones around individual colonies growing on Provasoli's enriched sea water medium supplemented with 0.27 mg/liter KI (PESI), 1% sodium alginate, 2% agar as previously described [13]. The same approach was used to isolate cellulase secreting bacteria, with 0.1% CMC replacing the alginate. All isolated colonies were restreaked several times to ensure the isolation of pure cultures. Stock cultures were maintained on slants containing either 1% ALG or 0.1% CMC and PESI solidified with 2% agar. For long-term storage, 3 vol. of an exponential culture in one of the above media minus the agar were mixed with 1 vol. of sterile glycerol and stored at −70°C.

For comparing growth rates and yields on different liquid media, 10 ml volumes of PESI containing either 0.1% ALG, 0.1% agar, or 1% CMC were inoculated with 0.1 ml of a suspension of cells prepared from a loop of 24 h (for ALG cultures) or 48 h (for CMC cultures) agar cultures suspended in 0.5 ml of PESI, sea water medium [13]. Cultures were incubated at room temperature without shaking. At 24 and 48 h the contents were mixed briefly by shaking and absorbance at 600 nm was determined as a measure of turbidity directly in the culture tubes using a Coleman 44 spectrophotometer. For providing quantitative growth data, fresh cultures were inoculated from these liquid cultures every 24 h to ensure depletion of residual substrates that might have been carried over from the original inocula.

For the analysis of the growth rates, substrate utilization, and product formation on alginate medium, 100 ml volumes of exponential cultures growing on PESI containing 0.1% ALG (with shaking) were inoculated into 1000 ml of the same medium in 2.8 liter fernbach flasks. The cultures were incubated at room temperature with shaking at 120 rpm on a G-10 Gyrotory shaker (New Brunswick Scientific). Samples (1–2 ml) were removed at various times to assay for turbidity at 600 nm (Gilford spectrophotometer, 1.00 cm cell), total uronic acid, and unsaturated uronides with the thiobarbituric acid assay as described below. For the preparation of enzymes, 1 liter cultures were grown in fernbach flasks to late exponential phase as above.

Fractionation and chemical analysis of alginate and its degradation products

For the characterization of products derived from the medium, 100 ml of medium were removed

at 6 and 24 h, centrifuged at 12 000 × *g* for 10 min at 2°C to remove cells, and the supernatant solution was mixed with an equal volume of cold 100% ethanol. After standing in the cold overnight, the polyuronide precipitate was collected by centrifugation (1600 × *g* for 10 min at 4°C) and dissolved in water. The supernatant containing the ethanol-soluble fraction was concentrated in vacuo at 45°C to less than 10 ml, and was then brought to a measured volume of 12–15 ml with water.

Total uronic acid equivalents were determined for medium samples and purified fractions with the methods of Blumenkrantz and Asboe-Hansen [1] and Dubois et al. [6], using D-mannuronolactone as a standard. Unsaturated equivalents generated by the lyase reactions were determined by absorbance at 232 nm [12–16] and by the thiobarbituric acid reaction with β-formylpyruvate generated upon periodate oxidation of the unsaturated uronides [18], using 2-keto-3-deoxyoctulosonic acid as a standard and measuring the chromogen at 548 nm.

Chromatographic analysis of alginate degradation products

Limit polymers obtained from the digestion of native alginate were purified by gel filtration on a Bio-Gel P-2 (Bio-Rad Laboratories) column (2.5 × 133 cm) preequilibrated and eluted with 0.1 M NH_4HCO_3. Fractions were assayed for uronic acid, TBA-reactive compounds generated upon periodate treatment, and absorbance at 232 nm. The polymer peak eluting in the void volume was lyophilized to remove water and NH_4HCO_3. Analytical determinations of alginate degradation products containing less than seven uronic acid residues and an unsaturated residue were made as previously described [16] using reverse-phase ion-pairing HPLC on a C-18 column and measuring absorbance at 232 nm. With the exception of the absorbance detector (Gilson Holochrome), all of the components of the chromatographic system were obtained from Waters Associates. An 8 mm μbondapak 10 μ C-18 cartridge column in a Z-module compression unit was preceded by a RCSS C-18 Guard-PAK and eluted isocratically with 10% CH_3CN/0.1 M sodium phosphate (pH 6.5)/0.01 M tetrabutylammonium hydroxide. The system was operated with the combination of a model 721 controller, 710B injector, 6000A pump, and 730 data analyzer. The column was calibrated with standards of unsaturated dimeric, trimeric, tetrameric, and pentameric oligomannuronans derived from the degradation of poly(ManA) by the partially purified poly(ManA)-specific extracellular alginate lyase from the ALG-A organism [16].

Purification and measurement of alginate lyase activity.

The extracellular poly(ManA) specific lyase from the ALG-A isolate was partially purified from the medium of late exponential phase cultures as previously described [16]. This preparation showed a single activity band when subjected to native gel electrophoresis (T. Romeo, Ph.D. dissertation, 1986) and had a specific activity of 6640 units/mg protein.

The extracellular alginate lyase activity from the ALG-G isolate was obtained from the medium of exponential phase cultures after removing the cells by centrifugation at 12 000 × *g* for 10 min. The medium was concentrated 52-fold using a Millipore Pellicon cassette system and a polysulfone (PTGC) membrane with a 10KDa cutoff.The concentrated medium was subjected to two sequential concentration/dialysis steps using an Amicon YM10 membrane with positive pressure from N_2 at 20 lb/in^2 to provide an additional 18-fold concentration of enzyme in 0.5 M NaCl/0.1 M sodium phosphate (pH 7.0). The activity of this preparation toward native alginate was 3730 units/mg. The enzyme activity was stable at 0°C in this buffer but the activity was rapidly lost when dialysis was carried out against distilled water. Both the A and G extracellular enzymes were stable to freezing and thawing and were routinely stored at −70°C.

The alginate lyase actvities were determined in 0.5 M NaCl/0.025 M sodium phosphate buffer (pH 7.5) at room temperature. Product formation was measured at determined intervals with the TBA assay and/or continuously measured by following the absorbance at 232 nm on a Gilford model 2400 recording spectrophotometer with a 1.00 cm light

path. Both enzyme preparations were absolutely dependent upon the addition of exogenous substrate for activity. One unit of lyase activity is defined as that which will catalyze the formation of one nmol unsaturated residue per min at 25°C.

RESULTS

Utilization of alginate, carboxymethylcellulose, and agar by bacteria associated with Sargassum species

Since the structural polysaccharides of the cell walls and matrices of *Sargassum* tissues include cellulose as well as alginate, bacteria which were initially selected for their ability to grow on alginate were evaluated for their ability to grow in PESI sea water medium containing CMC as the carbon source. As shown in Table 1, several alginase-secreting bacteria grew well on the alginate-supplemented medium but showed little or no growth on the same medium with either 1.0% CMC or 0.1% agar as carbon sources. On the other hand, three isolates which were selected for their ability to grow on solid (2% agar) PESI medium containing 0.1% CMC were able to grow well or even better on the PESI liquid medium with either 0.1% ALG or 0.1% agar than with 1% CMC as carbon sources. These latter isolates, designated CMC-A, CMC-B, and CMC-C, all showed negligible growth on 0.1% CMC in PESI, and only a slight turbidity was observed for the CMC-B and CMC-C isolates on medium containing 1% CMC. Both the CMC-B and CMC-C isolates effected an obvious and rapid decrease in the viscosity of the 1% CMC medium, indicative of the secretion of endolytic cellulases by these bacteria.

All of the isolates obtained from growth on CMC medium (containing 2% agar) showed significant growth on liquid medium when agar was provided as the carbon source at 0.1%, while none of the alginase – secreting isolates that were originally selected for growth on medium containing 1% ALG and 2% agar showed any growth when agar alone was provided as the carbon source. All of the CMC isolates effected the appearance of obvious depressions on solid PESI medium containing

Table 1

Comparison of alginate, agar, and carboxymethylcellulose utilization by *Sargassum*-associated isolates

Cultures are designated as ALG or CMC if they were originally isolated on agar media containing either 1% alginate or 0.1% CMC, respectively. Absorbance values at 600 nm were determined as a measure of turbidity directly in 18 × 150 mm culture tubes containing 10 ml of PESI medium with different carbon sources after incubating cultures at room temperature for 24 h. Values reported are the mean from two separate experiments, along with the standard deviation.

	Absorbance 600 nm		
Isolate	0.1% ALG	0.1% AGAR	1.0% CMC
SFFB 080483 ALG-A	0.150 ± 0	0.007 ± 0.002	0.001 ± 0.001
SNFB 080483 ALG-B	0.779 ± 0.004	0.010 ± 0.002	0.002 ± 0
SNFB 080483 ALG-C	0.163 ± 0.002	0.012 ± 0.007	0.005 ± 0.002
SNFB 080483 ALG-D	0.170 ± 0.030[a]	0.011 ± 0.001	0.001 ± 0.001
SFFB 080483 ALG-F	0.178 ± 0.013[a]	0.012 ± 0	0.001 ± 0.001
SFFB 080483 ALG-G	0.043 ± 0.008	0.005 ± 0.005	0.007 ± 0.002
SFFB 080483 ALG-H	0.161 ± 0.030[a]	0.011 ± 0.001	0.003 ± 0.003
SFBP 022685 CMC-A	0.105 ± 0.030[a]	0.148 ± 0.028[a]	0.003 ± 0.003
SFBP 022685 CMC-B	0.136 ± 0.035[a]	0.147 ± 0.025[a]	0.023 ± 0.008[b]
SFBP 022685 CMC-C	0.144 ± 0.034[a]	0.125 ± 0.004[a]	0.039 ± 0.014[b]

[a] Cultures showed extensive clumping.

[b] Cultures showed a dramatic decrease in the viscosity of the culture medium containing 1% CMC.

either 0.1% CMC and 2% agar or 2% agar alone. Continued growth of the CMC-B and CMC-C isolates on 1% CMC medium with shaking for an additional 24–48 h resulted in absorbance values (600 nm) of 0.2, compared to values of 0.4 which could be achieved when 0.1% alginate was provided as the sole carbon source (J.F. Preston and D.R. Preston, unpublished results). Microscopic examination revealed no obvious differences between the CMC and the ALG isolates, all of which were Gram-negative motile rods.

Growth, alginate utilization, and accumulation of alginate degradation products by ALG-A and ALG-G cultures.

Two of the fermentative alginase-secreting bacteria, ALG-A and ALG-G, that were originally isolated from actively growing tissues of *S. natans* and *S. fluitans* and characterized with respect to metabolic and morphological properties [13] were chosen for further evaluation of their ability to degrade native alginate in culture. Fig. 1 describes the growth curves for these bacteria on PESI, 0.1% ALG medium, with the analysis of total uronic acid and unsaturated residues as a function of time. The ALG-A isolate (Fig. 1a) showed an estimated doubling time of 5 h and by the end of the exponential phase of growth provided 1.85 g of wet cell weight per 1 g alginate initially present; for comparable conditions, the ALG-G isolate gave a doubling time of 8 h or longer and provided 0.57 g of wet weight.

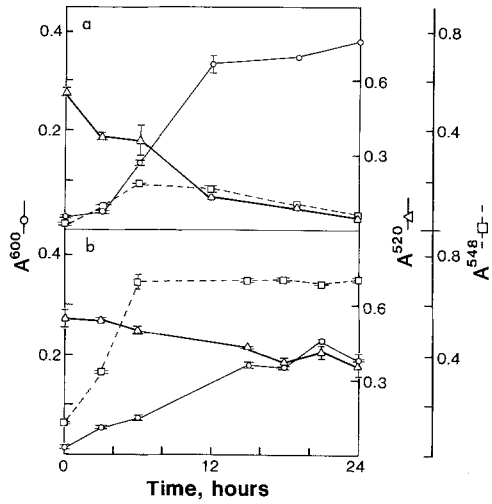

Fig. 1. Growth of ALG-A (a) and ALG-G (b) isolates in liquid alginate cultures. Cultures were assayed at indicated time points for turbidity (A^{600}) as a measure of growth, total uronic acid equivalents (A^{520}), and unsaturated residues with the TBA assay (A^{548}) as described in the text. Values for each parameter were obtained from duplicate cultures and averaged with ranges indicated by the bars.

Table 2

Distribution of uronic acid and unsaturated residues in ethanol-soluble and -insoluble fractions obtained from the media of ALG-A and ALG-G cultures

Uronic acid equivalents are given for 100 ml of medium. Unsaturated residues, Δ, determined by the TBA assay are given for 100 ml of medium.

Isolate	Time (h)	C_2H_5OH-soluble			C_2H_5OH-insoluble		
		Uronate (μmol)	Δ (μmol)	DP	Uronate (μmol)	Δ (μmol)	DP
ALG-A	6	53	21	2.6	195[a]	18[a]	11
ALG-A	24	34	13	2.6	19	0.52	36
ALG-G	6	319	165	1.9	24[b]	0.46[b]	53
ALG-G	24	213	135	1.6	2.1	0.016	131

[a] Equivalents of uronic acid and unsaturated residues were calculated from values obtained on unfractionated medium minus values obtained for the ethanol supernatants, and are given for 100 ml of medium.

[b] Equivalents of uronic acid were determined with the phenol-sulfuric acid method and the unsaturated residue equivalents with the TBA assay. These measurements were made after fractionation of the ethanol pellet fraction on Bio-Gel P-2 and are calculated for 100 ml of medium.

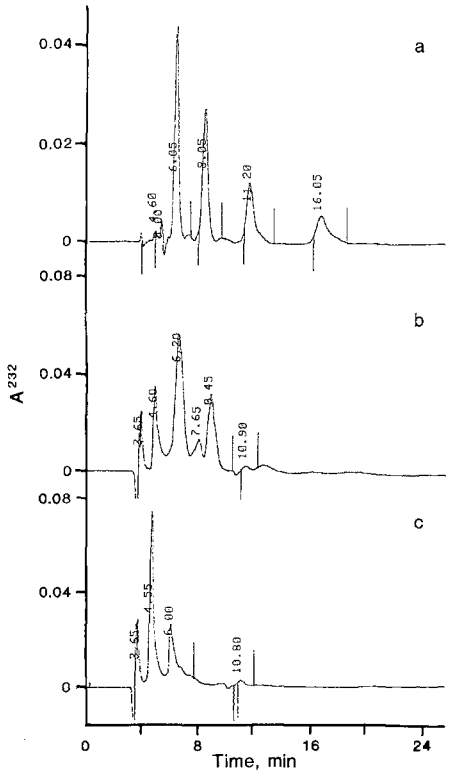

Fig. 2. HPLC analysis of ethanol-soluble fractions obtained from 6 and 24 h cultures of the ALG-A isolate. A flow rate of 1 ml/min was used with detection of absorbance at 232 nm as described in the text. Concentrations of uronic acid and unsaturated equivalents are given in Table 2. For a, 0.01 ml of a mixture of unsaturated mannuronide standards including a dimer, trimer, tetramer, and pentamer, each at 0.2 mg/ml, was injected. b is for the injection of 0.01 ml of the 6 h fraction containing 18 nmol unsaturated equivalents. c is for the injection of 0.01 ml of the 24 h fraction containing 10 nmol unsaturated equivalents.

The lower growth yield found for the ALG-G isolate was accompanied by the disappearance of only 13% of the total uronic acid equivalents (measured in the whole medium) initially supplied as alginate, along with the accumulation by 5 h of a significant and constant level of unsaturated residues as determined with the TBA assay (Fig. 1b). The ALG-A isolate consumed 90% of the uronic acid equivalents by 24 h, and at no time accumulated the large levels of unsaturated residues observed for the ALG-G organism.

The comparison of these two bacteria and their utilization of alginate is given in Table 2, with the analyses of the polymer fractions precipitated by 50% ethanol in the cold, and the smaller oligouronides, expected to have degree of polymerization (DP) values of less than 10, in the fraction soluble in 50% ethanol. The molar ratios of unsaturated residues, used as a measure of nonreducing terminal ends, to the uronic acid equivalents provided estimates of the DP values for alginate degradation products in 100 ml samples of media obtained from 6 and 24 h cultures of both organisms. The ALG-G isolate showed very high levels of unsaturated residues in the ethanol soluble fraction at both 6 and 24 h time points, with an average DP of 1.6–1.9. The ALG-A organism shows much lower levels of oligouronides in the ethanol-soluble fraction, with an average DP of 2.6 at both 6 and 24 h. For the 6 and 24 h time points, the ALG-A organism had taken up 50–89% of the uronic acid equivalents, respectively, while the ALG-G isolate had taken up 31 and 57%.

The characterization of the alginate in the ethanol-soluble fractions from the media was extended with HPLC analyses presented in Figs. 2 and 3. With a reverse-phase ion-pairing isocratic system previously used to quantify the oligomannuronide products formed during the depolymerization of

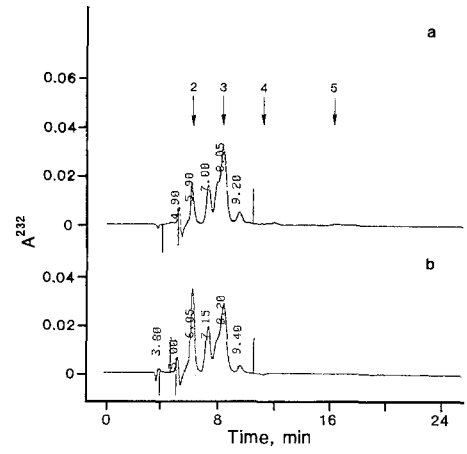

Fig. 3. HPLC analysis of ethanol-soluble fractions obtained from the 6 and 24 h cultures of ALG-G isolate. Conditions are those described in the legend for Fig. 2. Arrows identify the elution positions for the unsaturated oligomannuronide standards. a is for the injection of 0.02 ml of the 6 h fraction containing 20 nmol of unsaturated equivalents. b is for the injection of 0.02 ml of the 24 h fraction containing 21 nmol of unsaturated equivalents.

poly(ManA) by the poly(ManA)-specific extracellular lyase from the ALG-A isolate [16], oligomers ranging from dimers through pentamers were well resolved (Fig. 2a). A sample from the 6 h medium (Fig. 2b) from the ALG-A isolate was resolved into significant peaks corresponding to a dimer (*RT* of 6.2) and trimer (*RT* of 8.45), with smaller amounts of a tetramer (*RT* of 10.9); the 24 h medium (Fig. 2c) showed the presence of a dimer (*RT* of 6.0) with a possible trace of trimer and tetramer remaining. The analogous analyses made with the ethanol-soluble fractions from the ALG-G isolate are presented in Fig. 3 and show that significant quantities of the dimer (*RT* of 5.9–6.0) and trimer (*RT* of 8.05–8.2) accumulate, with the amount of the dimer increasing by 24 h. The A^{232} peaks eluting at the other positions are presumed to be compounds other than unsaturated uronides, since these were resolved from the dimer and trimer uronide peaks upon gel filtration on Bio-Gel P-2 and were not detected with the TBA assay (data not shown).

Selective degradation of alginate by extracellular alginate lyases of ALG-A and ALG-G bacteria

The partially purified extracellular alginate lyase from the ALG-A isolate, which has been shown to be specific for the poly(ManA) portion of alginate [16], and a crude fraction from the medium of the ALG-G isolate, which was prepared by concentration and dialysis, were evaluated upon sequential addition to alginate for their ability to degrade alginate and accumulate limit products reflecting the specificities of the respective enzymes involved. Fig. 4 shows the kinetic response of the ALG-A enzyme followed by the ALG-G enzyme of A^{232} as a measure of unsaturated residues formed by the lyase-catalyzed reaction. Exposure of native alginate at 0.3 mg/ml to 0.06 ml of the ALG-A enzyme for 120 min led to the formation of an apparent limit product with an A^{232} of 0.68. The addition of 0.03 ml of the A enzyme at 90 min did not change the rate, indicating that the decrease in the rate that occurred later in the reaction was not a result of a loss of activity of the A enzyme during the reaction. The addition of the ALG-G enzyme at 120 min led to a rapid increase in the A^{232} with the formation of an additional set of limit products by 360 min. The increase in A^{232} during the reaction was paralleled by an increase in the formation of products that were detected with thiobarbituric acid after periodate treatment, validating the use of absorbance measurements to follow the lyase reaction.

The limit products that were formed with the in vitro reaction were analyzed by HPLC for comparison with those detected in the media of the ALG-A and ALG-G bacteria. Fig. 5a shows an HPLC profile for the limit products formed by the action of the ALG-G lyase activity alone on native alginate. Both a dimer (*RT* of 5.83) and a trimer (*RT* of 7.5) were present, as well as a small amount of tetramer (*RT* of 11.04) and a component with an *RT* of 6.66. The presence of a component, possibly a pentamer, with an *RT* value greater than that of the tetramer was noted, although it was not present in

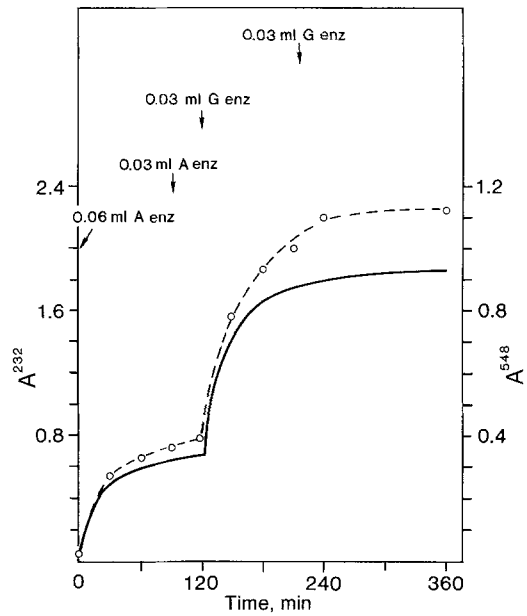

Fig. 4. Sequential treatment of native alginate with ALG-A and ALG-G extracellular lyases. Reactions were followed with continuous measurement of A^{232} (——) of a 3 ml reaction mixture containing 0.03% alginate in 0.5 M NaCl, 0.025 M sodium phosphate buffer (pH 7.5) at 29°C. Samples (0.050 ml) were removed at the indicated times and assayed for unsaturated equivalents with the TBA method (O--O). The reaction was initiated with the addition of 0.06 ml containing 7.56 units of the ALG-A enzyme, with the later addition of an addition of 0.03 ml at 90 min. At 120 and 240 min, 0.03 ml of the ALG-G lyase preparation was added.

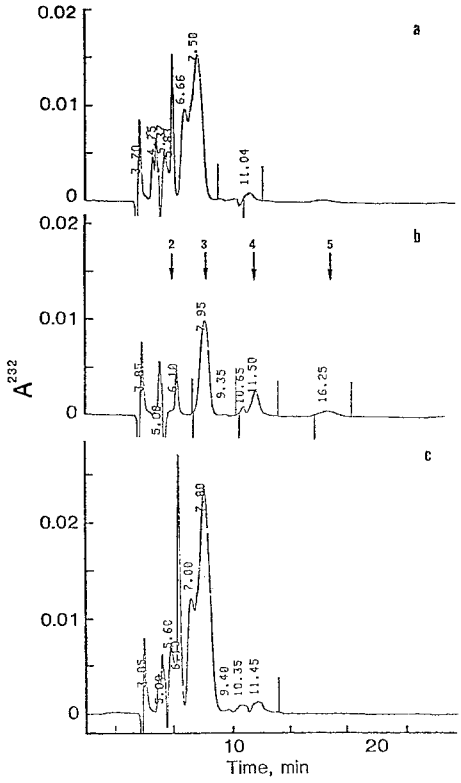

Fig. 5. HPLC analyses of in vitro alginate degradation reaction. Conditions are the same as those described for Fig. 2, and the arrows identify the elution positions of the oligomannuronide standards. a is for the injection of 0.02 ml of a 180 min reaction initially containing 0.02% alginate and 3.3 units of the ALG-G enzyme preparation in a 1.0 ml reaction mixture. b and c are for the injections of 0.02 ml of the reaction described in Fig. 4 at 120 and 240 min, respectively.

sufficient quantity to allow its integration. The ALG-A enzyme (Fig. 5b) formed mainly the trimer as limit product at 120 min, with smaller quantities of dimer and tetramer, and a detectable quantity of pentamer (*RT* of 16.25). The sequential action of the A followed by the G enzymes on alginate led to the formation of limit products (Fig. 5c) that were analogous to those found for the action of the G enzyme(s) alone, with the difference that a greater percentage of the dimer appeared. The pentamer that was present after treatment with A enzyme alone was absent after treatment with the G enzyme(s).

DISCUSSION

The isolates selected as alginase-secreting bacteria from actively growing tissues of *Sargassum* have been previously characterized as Gram-negative polarly flagellated rods with either oxidative (ALG-B,C,F) or fermentative (ALG-A,D,G,H) growth potential. Based on DNA base composition, the oxidative isolates have been assigned to the genus *Alteromonas,* while the fermentative isolates most closely fit into the genus *Photobacterium,* although their percent GC values of 45.4–47.4 are higher than any species currently assigned to this genus [13]. All of these isolates failed to grow on PESI sea water media with either CMC or agar provided as the carbon sources, and the property for the synthesis and/or secretion of extracellular alginate lyases is therefore not linked to the synthesis and/or secretion of agar- and cellulose-degrading enzymes.

In the cases where bacteria were isolated from the *Sargassum* tissues on the basis of their ability to grow on CMC, enzymes capable of degrading alginate and agar as well as CMC were secreted, allowing the possibility that the expression of these activities may be linked in at least some bacteria. Further studies with the CMC-B and CMC-C isolates indicate that they show very slow growth when incubated with crystalline cellulose as the sole carbon source, yet they secrete cellulases (CMCases) in levels comparable to those produced when grown with 1% CMC as the sole carbon source. (J.F. Preston and D.R. Preston, unpublished results). The number of bacteria isolated by growth on CMC medium was much less than the number isolated from the same amount of sample on alginate medium, and the cellulase-secreting bacteria have yet to be taxonomicaly assigned. These may be analogous to those isolated from *Fucus* tissues, which were able to use a number of different carbohydrate polymers [14].

The growth of the fermentative ALG-A and ALG-G isolates on alginate liquid medium demonstrated a clear distinction between these organisms, with the A isolate growing more rapidly and producing a greater mass yield. The accumulation of

large amounts of unsaturated uronides by the G isolate is correlated with its inability to take more than a fraction of the alginate as measured by the disappearance of total uronic acid equivalents. It is apparent that this bacterium is able to degrade nearly all of the alginate in the medium to unsaturated derivatives that are soluble in cold 50% ethanol, and that analysis of this fraction by HPLC indicates that these are predominantly dimers and trimers. The G isolate therefore appears to have a limitation in the ability to take up certain unsaturated dimeric and trimeric derivatives that are readily formed by its secreted alginate lyase(s), and the further characterization of these compounds is intended.

The action of the poly(ManA) specific lyase secreted by the ALG-A bacterium on native alginate produced a limit that included a size distribution of the smaller (less than ten residues) unsaturated oligouronides that is analogous to that produced by the action of this same enzyme on poly-D-mannuronate [16]. By the time the rate of double bond formation levels off, e.g., 90 min in Fig. 4, the native alginate has been degraded to products that include a major fraction that is eluted in the void volume of Bio-Gel P-2 and is sufficiently large to preclude its elution from the C-18 column during reverse-phase ion-paired HPLC. This fraction would presumably include the poly(GulA)-containing portions of the alginate, since the ALG-A extracellular enzyme is specific for poly(ManA) and showed no activity with poly(GulA). It is not considered coincidental that the medium for 6 h cultures of the ALG-A organism contained an ethanol-insoluble fraction with a DP value of 10, as the secreted poly(ManA) lyase would be expected to generate limit polymers enriched in the L-guluronate residues. The later degradation of this fraction in the stationary phase may reflect the release during cell leakage and/or lysis of intracellular enzymes that are normally active on poly(GulA) as well as poly(ManA) [15].

Both the ALG-A and ALG-G organisms represent fermentative bacteria that appear to operate in a commensal and possibly mutualistic relationship with each other and/or other alginate-degrading bacteria associated with actively growing tissues of species of *Sargassum*. It will be of interest to evaluate additional species of alginate lyase-secreting bacteria associated with the brown algae to determine whether the naturally occurring populations are comprised of species which are interdependent rather than competitive in their reliance upon alginate as a carbon and energy source. The ability of these and other bacteria to selectively degrade alginate with the production of specific products at present identifies them as candidates for further consideration as sources of specific alginate modifying enzymes.

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