APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1982, p. 754–756
0099-2240/82/090754-03$02.00/0
Copyright © 1982, American Society for Microbiology
Vol. 44, No. 3

Isolation of Marine Bacteria Capable of Producing Specific Lyases for Alginate Degradation

R. SCOTT DOUBET AND RALPH S. QUATRANO*

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331

Received 22 March 1982/Accepted 18 May 1982

Alginate lyases (EC 4.2.2.3) were isolated from cultures of several marine bacterial isolates. The lyases were induced by native alginate and had activity toward both the mannuronic acid and the guluronic acid blocks of the alginate polymer. The guluronic acid-specific lyase was recovered from the medium, whereas the mannuronic acid-specific lyase was retained with the bacteria.

Our laboratory has isolated an unique marine bacterium that can use a wide variety of marine algal polysaccharides as sole carbon sources (18). We now have additional isolates with similar properties. This report is concerned with the ability of several of these bacterial isolates to produce enzymes that degrade alginate, one of the most prevalent of the brown algal cell wall polymers, and includes data concerning the location of the enzymes in the bacterial cultures and the components in the medium required for enzyme activity. We are interested in these enzymes as probes to release subsets of algal cell walls to study wall assembly during *Fucus* zygote development (19).

About 60% of the cell wall mass of the brown alga *Fucus* is composed of alginate (19), a linear glycuronan polymer of α-L-guluronic acid (G) and β-D-mannuronic acid (M). The primary structure of alginate is highly variable in terms of monomer ratios and distribution of monomers into homopolymeric blocks (GG or MM) or heteropolymeric blocks (MG) (5, 6). This variability in the primary structure is observed between species and within different parts of a single plant (8). The monomers of alginate are linked by 4-O-glycosidic bonds which can be chemically degraded by acid hydrolysis (7) or alkali-catalyzed β-elimination (10). These chemical treatments are likely to disrupt other polysaccharide linkages in the wall and are not specific for alginate linkages. However, alginate lyase enzymes (EC 4.2.2.3) are specific for alginate and act in a manner analogous to that of pectin transeliminases (1), making them suitable for use in the selective disruption of algal cell walls.

Bacterial isolates from *Fucus distichus* receptacles were inoculated from single colonies into liquid culture as previously described (18). The cultures were grown at room temperature for 18 to 20 h (the end of the log phase) and then centrifuged. The culture medium was assayed directly for released alginate activity. Bacterial pellets were washed twice with culture medium minus alginate, suspended in double-distilled water, and sonicated on ice for 40 s at 90 W with a Branson Sonifier 185W. The cell homogenate was centrifuged at 12,000 × *g* for 20 min. The supernatant of the sonicated pellet was assayed directly for alginase and represents the bound enzyme activity. Further purification of both types of enzyme showed that both were stable when filtered with a Millipore filter (0.45 μm), dialyzed against distilled water, and lyophilized. Both enzymes were active at room temperature in a pH range from 6.0 to 9.0 with Tris-phosphate buffer (data not shown).

The lyase assay (17) was performed at room temperature in a volume of 0.2 ml containing enzyme, 125 μg of sodium alginate, 100 mM Tris, 50 mM KCl, and 50 mM NaCl (pH 7.5). One unit of enzyme activity was defined as an increase in the absorbance at 550 nm of 1.0 unit per min by the thiobarbiturate colorimetric assay (20).

Alginates from *Macrocystis pyrifera*, obtained from Sigma Chemical Co., St. Louis, Mo., were routinely used as enzyme substrates and were the source of homopolymeric G and M blocks. G and M blocks were prepared by acid hydrolysis of high-molecular-weight alginates (8) and were determined by B. Larsen, Institute of Marine Biochemistry, Trondheim, Norway, using ^{1}H nuclear magnetic resonance spectroscopy, to be 97% G or M with average degrees of polymerization of 26 and 22, respectively. We confirmed the nuclear magnetic resonance analysis by circular dichroism (14).

When isolates were grown in media containing alginate as a carbon source, crude enzymes could be obtained from the culture filtrates or

Downloaded from https://journals.asm.org/journal/aem on 24 February 2022 by 2a01:4f9:3b:1ae9::

TABLE 1. Substrate preference of crude bacterial enzymes from 24-h cultures measured by the thiobarbiturate assay

Isolate	Lyase act (U)[a] of:					
	Released enzyme with:			Bound enzyme with:		
	G	M	M/G[b]	G	M	M/G
A_3	0.06	0.06	1.00	1.69	30.9	18.3
A_4	0.02	0.08	4.00	2.25	43.1	19.2
A_7	0.02	0.17	8.50	1.31	36.2	27.6
A_9	0.10	0.10	1.00	4.31	46.9	10.9
A_5	1.42	0.62	0.44	4.69	6.38	1.36
A_{12}	0.62	0.25	0.40	2.44	5.25	2.15
W_0	1.15	0.77	0.67	3.19	5.25	1.65
W_3	2.17	0.65	0.30	3.75	18.8	5.00

[a] One unit of lyase activity is defined as an increase in the absorbance at 550 nm of 1.0 unit per min, corrected for volumes and incubation time. Assay methods are described in the text.
[b] Ratio of the activity of the enzyme toward M substrate to that toward G substrate.

bacterial pellets. In all of our bacterial cultures, enzymes with lyase activity toward both M and G substrates were evident (Table 1).

Although enzymes capable of depolymerizing alginate have been obtained from a variety of sources, enzymes isolated from molluscs generally show a preference for M-rich substrates, whereas bacterial enzymes prefer G-rich substrates. The enzymes isolated from molluscs have been characterized as endo-M lyases (4, 15, 16) and a possible exo-G lyase (16). Cell-bound enzymes isolated from a variety of bacterial sources have been characterized as G lyases (3, 9, 11, 12) and a very weak M lyase (9). An extracellular G lyase has also been characterized from a bacterial source (2). Thus, with the exception of those isolates described in this report, lyases obtained from a number of bacterial genera clearly do not show high activity and specificity toward M block substrates.

Not only did our bacterial isolates display a high-activity enzyme specific for M blocks, but a definite trend in enzyme substrate preference for short (average degree of polymerization = 20) M or G blocks was noticed with respect to the enzyme source. Bound enzymes showed a preference for M-rich substrates, whereas released enzymes showed a preference for G-rich substrates (Table 1). In addition, most isolates were dependent upon alginate in the medium for the recovery of any lyase activity (e.g., W_0 and A_3, Table 2). Acquisition and loss of lyase activity was noticed on the first transfer into the appropriate medium with or without alginate. One exception was noticed. Isolate W_3 produced and released a lyase when yeast extract was present with either alginate or glucose in the medium. In the latter case, yeast extract may contain a polysaccharide similar enough to alginate to act as an inducer. When isolate W_3 was grown on glucose and NH_4NO_3, growth was identical to that in other media, but the release of lyase activity was reduced over 20-fold. Thus, W_3 has the ability to release an alginate lyase without alginate in the medium, a characteristic which has helped in purification of the released enzyme from the viscous alginate in the medium (unpublished data).

In summary, this report is the first to demonstrate a highly active M-degrading lyase from a bacterial source, the location of M- and G-degrading lyases in a bound or released form, and the environmental control of enzyme induc-

TABLE 2. Alginate lyase activity from isolates grown on media with various carbon and nitrogen sources

Isolate	Enzyme source[a]	Medium[b]		Lyase act (U)[c]
		Carbon source	Nitrogen source	
W_0	Released	Alg	YE	1.46
		None	YE	0.09
A_3	Bound	Alg	YE	13.2
		Glu	YE	0.00
W_3	Released	Alg	YE	1.99
		None	YE	1.92
		Glu	YE	1.72
		Glu	NH_4NO_3	0.09

[a] Enzymes isolated from the medium or from bacteria were extracted from the same mass or volume and assayed with high-molecular-weight alginates as substrates according to the procedures described in the text and Table 1.
[b] All media contained the basal salt mixture (18). In addition, a nitrogen source (0.1% yeast extract [YE] or 0.2% NH_4NO_3) with or without a carbon source (0.1% alginate [Alg] or 0.5% glucose [Glu]) was added. The amount of growth in the cultures was the same when grown on the different media.
[c] See Table 1 for definition of unit.

Downloaded from https://journals.asm.org/journal/aem on 24 February 2022 by 2a01:4f9:3b:1ae9::

tion. The trend in these isolates to release G-degrading enzymes is interesting in that calcium-alginate gels are not formed in the presence of small G-rich oligouronides (13). The action of a G-degrading enzyme would possibly result in the disruption of alginate gels in the natural environment, releasing oligouronides for further bacterial metabolism of alginate.

LITERATURE CITED

1. **Albersheim, P., H. Neukom, and H. Deuel.** 1960. Splitting of pectin chain molecules in neutral solutions. Arch. Biochem. Biophys. **90**:46–51.
2. **Boyd, J., and J. R. Turvey.** 1977. Isolation of a poly-α-L-guluronate lyase from *Klebsiella aerogenes*. Carbohydr. Res. **57**:163–171.
3. **Davidson, I. W., I. W. Sutherland, and C. J. Lawson.** 1976. Purification and properties of an alginate lyase from a marine bacterium. Biochem. J. **159**:707–713.
4. **Elyakova, L., and V. V. Favorov.** 1974. Isolation and certain properties of alginate lyase VI from the mollusk *Littorina* sp. Biochim. Biophys. Acta **358**:341–354.
5. **Grasdalen, H., B. Larsen, and O. Smidsrød.** 1979. A P.M.R. study of the composition and sequence of uronate residues in alginate. Carbohydr. Res. **68**:23–31.
6. **Grasdalen, H., B. Larsen, and O. Smidsrød.** 1981. ^{13}C-NMR studies of monomeric composition and sequence in alginate. Carbohydr. Res. **89**:179–191.
7. **Haug, A., B. Larsen, O. Smidsrød.** 1967. Studies on the sequence of uronic acid residues in alginic acid. Acta Chem. Scand. **21**:691–704.
8. **Haug, A., B. Larsen, and O. Smidsrød.** 1974. Uronic acid sequence in alginate from different sources. Carbohydr. Res. **32**:217–225.
9. **Kashiwabara, Y., H. Suzuki, and K. Nisizawa.** 1969. Alginate lyases of pseudomonads. J. Biochem. (Tokyo) **66**:503–512.
10. **Kiss, J.** 1974. β-Eliminative degradation of carbohydrates containing uronic acid residues. Adv. Carbohydr. Chem. Biochem. **29**:229–303.
11. **Min, K. H., S. F. Sasaki, Y. Kashiwabara, and K. Nisizawa.** 1977. Substrate specificity of endo-polyguluronide lyase from pseudomonas sp. on the basis of their kinetic properties. J. Biochem. (Tokyo) **81**:547–554.
12. **Min, K. H., S. F. Saskai, Y. Kashiwabara, H. Suzuki, and K. Nisizawa.** 1977. Multiple components of endo-polyguluronide lyase of pseudomonas sp. J. Biochem. (Tokyo) **81**:539–546.
13. **Morris, E. R., D. A. Rees, G. Robinson, and G. A. Young.** 1980. Competitive inhibition of interchain interactions in polysaccharide systems. J. Mol. Biol. **138**:363–374.
14. **Morris, E. R., D. A. Rees, and D. Thom.** 1980. Characterization of alginate composition and block structure by circular dichroism. Carbohydr. Res. **81**:305–314.
15. **Muramatsu, T., S. Hirose, and M. Katayose.** 1977. Isolation and properties of alginate lyase from the mid-gut gland of wreath shell *Turbo cornutus*. Agric. Biol. Chem. **41**:1939–1946.
16. **Nakada, H. I., and P. C. Sweeny.** 1967. Alginic acid degradation by eliminases from abalone hepatopancreas. J. Biol. Chem. **242**:845–851.
17. **Preiss, J., and G. Ashwell.** 1962. Alginic acid metabolism in bacteria. I. Enzymatic formation of unsaturated oligosaccharides and 4-deoxy-L-erythro-5-hexoseulose uronic acid. J. Biol. Chem. **237**:309–316.
18. **Quatrano, R. S., and B. A. Caldwell.** 1978. Isolation of a unique marine bacterium capable of growth on a wide variety of polysaccharides from macroalgae. Appl. Environ. Microbiol. **36**:979–981.
19. **Quatrano, R. S., and P. T. Stevens.** 1976. Cell wall assembly in *Fucus* zygotes. I. Characterization of the polysaccharide components. Plant Physiol. **58**:224–231.
20. **Weissbach, A., and J. Hurwitz.** 1959. The formation of 2-keto-3-deoxyheptonic acid in extracts of *Escherichia coli* B. I. Identification. J. Biol. Chem. **234**:705–709.

Downloaded from https://journals.asm.org/journal/aem on 24 February 2022 by 2a01:4f9:3b:1ae9::