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Lysis of Bacterial Cell Walls by an Enzyme Isolated from a Myxobacter

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ABSTRACT

ENSIGN, J. C. (University of Illinois, Urbana), AND R. S. WOLFE. Lysis of bacterial cell walls by an enzyme isolated from a myxobacter. J. Bacteriol. **90**:395-402. 1965.—An exoenzyme which lyses intact cells, heat-killed cells, and cell walls of *Arthrobacter crystallopoietes* was purified 60-fold from the growth liquor obtained from a myxobacter (strain AL-1). The lytic enzyme was produced during growth of the organism in a number of complex media, the maximal amount of enzyme being produced in yeast extract broth. The purified enzyme lysed at different rates a number of gram-positive bacteria. With the exception of *Rhodospirillum rubrum*, *Spirillum itersonii*, and *S. serpens*, the gram-negative bacteria tested were not attacked.

We have described recently our studies on the nutritional control of morphogenesis in *Arthrobacter crystallopoietes* (Ensign and Wolfe, 1964*a*). To obtain evidence related to cell-wall structure, attempts were made to isolate from soil organisms which would lyse cells of *A. crystallopoietes*. This report describes the isolation and growth characteristics of a myxobacter (strain AL-1) which produces an extensive zone of lysis on a lawn of *A. crystallopoietes*. Conditions for maximal production of the lytic enzyme, procedures for its partial purification, and a survey of its lytic activity against different microorganisms are presented. A brief report of some of these findings has appeared (Ensign and Wolfe, 1964*b*).

MATERIALS AND METHODS

Isolation and maintenance of cultures. To prepare agar lawns of *A. crystallopoietes*, each plate of sterile medium which contained 0.5% peptone and 1.0% agar, was overlaid with 5.0 ml of medium which contained 1.0% agar, 0.5% peptone, and cells of *A. crystallopoietes* (about 10^9 cells per milliliter of medium). When solid, this medium (medium A) was streaked with a loopful of an aqueous extract of soil and was incubated at 30 C. Cells from colonies which exhibited zones of lysis were isolated in pure culture upon medium B which contained 1.5% agar and cells of *A. crystallopoietes* (10^9 cells per milliliter of medium). Cells used as substrate in medium B were centrifuged from the growth medium (Ensign and Wolfe, 1964*a*), and were washed aseptically by suspending the cells in an equal volume of water. After centrifugation, the cells were suspended aseptically in a smaller volume of water so that, when added to the medium, the final concentration was about 10^9 cells per milliliter. Cultures of lytic organisms were maintained by weekly transfer on medium B; at each transfer, an isolated colony which was surrounded by a zone of lysis was picked and transferred to sterile medium. An incubation temperature of 30 C was employed throughout the investigation.

Preparation of substrates. Except where specifically mentioned, assays of enzyme activity were performed with heat-killed suspensions of *A. crystallopoietes* as substrate. This organism was grown in 8-liter batches in the glucose-salts medium of Ensign and Wolfe (1964*a*). After harvesting by centrifugation, the cells were stored at −20 C. A 5-g amount of frozen cell paste was suspended in 50 ml of 0.025 M tris(hydroxymethyl)-aminomethane (Tris)-HCl buffer at *p*H 7.8, and the suspension was heated for 10 min in a water bath at 90 C. The cells were then cooled rapidly in an ice bath, centrifuged, washed once in 50 ml of water, and suspended in 0.02 M Tris-HCl buffer at *p*H 9.0. This suspension was adjusted to a concentration such that 1.0 ml when diluted with 4.0 ml of buffer gave a reading on the Klett colorimeter of about 500 at 660 mμ (an absorbancy of 1.0). The standard suspension of heated cells was stored at 4 C, a fresh batch being prepared each week.

Cell walls, which were employed as substrate in several experiments, were prepared by suspending 10 g (wet weight) of *A. crystallopoietes* in water to a final volume of 30 ml. A 10-g amount of no. 12 glass beads (Minnesota Mining and Manufacturing Co., St. Paul, Minn.) was added, and the suspension was subjected to 30 min of treat-

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ment in a Raytheon 10-kc sonic oscillator; the temperature of the cell-bead mixture was maintained below 15 C. After sonic treatment, the glass beads were removed by use of a coarse sintered-glass filter, and the beads were washed with two successive 5-ml portions of water, each filtrate being collected. The combined filtrates were centrifuged for 10 min at 500 × *g*; the supernatant fluid was removed and was centrifuged for 20 min at 13,000 × *g*. The supernatant liquid was decanted and discarded. The pellet consisted of a tightly packed bottom layer of predominantly intact cells overlaid with a flocculent, gray layer of both cell walls and intact cells. The upper layer was washed carefully from the tube with a gentle stream of water and was centrifuged at 13,000 × *g* for 20 min. After 12 similar water washes, a preparation was obtained which had an absorbancy at 660 mμ of 2.0 and in which only an occasional intact cell could be found by extensive microscopic analysis.

Procedures for obtaining larger batches of growth liquor. A 500-ml Erlenmeyer flask containing 100 ml of 1.0% yeast extract (medium C) was inoculated with a loopful of cells from a colony of the lytic organism. The flask was shaken at 30 C for 36 hr, after which time a 3-liter flask containing 600 ml of medium C was inoculated with 30 ml of the starter culture. After shaking at 30 C for 24 hr, the contents of this flask were used to inoculate a 12-liter flask containing 6 liters of medium C. The flask was vigorously aerated at 30 C by the passage of sterile air through a sintered-glass sparger at the bottom of the flask. Four drops of Antifoam A (Dow-Corning Corp., Midland, Mich.) were added prior to sterilization of the medium.

Assay procedures. The level of the lytic enzyme in growth liquor was determined by measuring the extent of lysis of a heat-killed suspension of *A. crystallopoietes*. A 2-ml amount of growth liquor, from which the cells had been removed by centrifugation, was added to a test tube (14 by 125 mm); 2 ml of 0.05 M Tris-HCl buffer at *p*H 9.0 were added, followed by 1.0 ml of the heat-killed cell suspension. The turbidity of the tube was immediately determined with a Klett-Summerson colorimeter at 660 mμ. The tube was then placed in a water bath (38 C), and the turbidity was again determined after exactly 15 min. Activities of purified enzyme preparations were similarly determined, water being added to bring the volume to 2.0 ml before adding buffer and substrate. One unit of lytic activity is defined as a change in absorbancy of 0.001 at 660 mμ of a heat-killed suspension of *A. crystallopoietes* in 15 min. Specific activity is defined as the number of units per milligram of protein. Protein was determined as described by Lowry et al. (1951).

Proteolysis was assayed by a procedure adapted from that of Ghuysen and Strominger (1963) in which the release of free amino groups is measured with dinitrofluorobenzene (DNFB). To 1.0 ml of a 0.2% solution of bovine serum albumin in a test tube (14 by 125 mm) were added a suitable sample of purified enzyme or dialyzed growth liquor, 1.0 ml of 0.02 M Veronal buffer at *p*H 9.0, and water to a total volume of 2.5 ml. At zero-time and after incubation at 38 C for 1 hr, a 0.1-ml sample was removed and added to 1.0 ml of 1.0% $Na_2B_4O_7 \cdot 10H_2O$ in a small Pyrex test tube. The tubes were heated for 1 min at 95 C to stop enzyme activity. A 0.1-ml amount of a 0.01 M solution of DNFB in ethyl alcohol was added to each tube, and the tubes were placed in a water bath (60 C) for 30 min; after this time, 4.5 ml of 2 M HCl were added to each tube, and the absorbancy at 420 mμ was determined in a Beckman DU spectrophotometer. A blank containing water and enzyme but no albumin, and a blank with albumin without enzyme, were similarly assayed. The assay readings were corrected for the blank values. One unit is defined as the amount of proteolytic activity yielding an increase in absorbancy of 0.001 at 420 mμ in 1 hr, and specific activity is expressed as units per milligram of protein.

RESULTS

Organism. The lytic organism grew abundantly on agar lawns of living or heat-killed cells of *A. crystallopoietes*. Colonies on the lawns were visible 24 to 36 hr after streaking, and after 5 or 6 days of growth were surrounded by zones of lysis which reached diameters of about 2.5 cm. These colonies were very mucoid, faintly yellow, round and flat, and were surrounded by a thin halo of cells extending 3 to 4 mm from the colony edge. The organism grew well on nutrient agar, producing bright-yellow colonies. On inverted plates of yeast extract-agar (1% yeast extract, 1.5% agar), two or more closely spaced colonies often coalesced, and during subsequent growth formed large drops of slime which fell from the center of the colony to the lid of the plate.

A plate containing 0.5% peptone and 1.5% agar was inoculated with a single heavy streak of *A. crystallopoietes*. After 48 hr, a thick growth having developed, one end of the streak was inoculated with a loopful of the lytic organism; after 7 days, the lytic organism had grown along the entire length of the streak, a distance of 65 cm. The lytic organism is a gram-negative rod, has rounded ends, and is 0.5 to 0.8 μ wide by 3 to 4 μ long. Observations of slide cultures and wet mounts by use of a phase-contrast microscope revealed the cells to be slightly flexuous, showing a creeping motility. No flagella were observed in electron micrographs of metal-shadowed cells.

The characteristics of copious slime production and creeping motility led us to believe that the organism is a member of the Myxobacterales. In no instance was fruiting-body formation observed; this was true when the organism was grown on a number of different media, including

TABLE 1. *Comparison of the maximal level of lytic activity obtained in various growth media**

Medium†	Substrate	Units of activity
1	Peptone	175
2	Peptone plus heat-killed cells	168
3	Proteose peptone	130
4	Neopeptone	145
5	Casitone	180
6	Casamino Acids	220
7	Yeast extract	480
8	Nutrient broth	110

* Each 500-ml Erlenmeyer flask contained 100 ml of medium and was shaken at 30 C; samples were removed during growth of the culture and were assayed for lytic activity as described in the text. Each flask was followed until the lytic activity remained constant.

† The sterile medium in each instance contained distilled water to which the substrate (Difco) indicated was added to a final concentration of 1%. Medium 2 contained, in addition to 1% peptone, 1.0 g (dry weight) of heat-killed cells of *Arthrobacter crystallopoietes*.

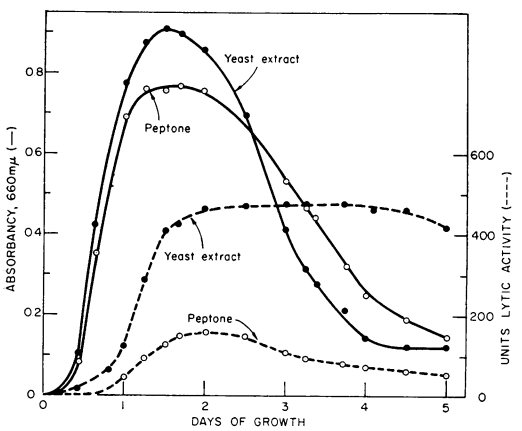

FIG. 1. *Comparison of cell growth and the formation of lytic activity in broth containing 1% peptone and in broth containing 1% yeast extract. See Materials and Methods for details of the assay technique.*

rabbit-dung pellets. Microcysts, characteristic of certain genera of myxobacteria, were not observed in either young or old cultures. Cultures older than 2 weeks contained only a few rods, much cell debris, and some spherical bodies of low refractility, resembling spheroplasts. Starch, cellulose, and chitin were not hydrolyzed. Since the organism is not readily classified, it is referred to as myxobacter strain AL-1. The myxobacter grew well in various liquid media, and the supernatant liquor of these cultures lysed living and heat-killed cells of *A. crystallopoietes*.

The levels of lytic activity produced in several complex liquid media were compared (Table 1). The cells grew well in each medium tested, with absorbancy varying between 0.70 and 0.95. Marked differences in the level of lytic factor were observed; yeast extract proved to be the superior medium. It was subsequently found that cells grown in 2 or 3% yeast extract broth produced essentially the same amount of lytic activity as those grown in 1% yeast extract, but the level was reduced more than half when cells were grown in 0.5% yeast extract. The addition of heat-killed cells of *A. crystallopoietes* to peptone broth did not increase the amount of lytic activity.

The time course of growth and of lytic-factor production in 1.0% solutions of peptone and of yeast extract is illustrated in Fig. 1. Growth in yeast extract slightly exceeded that in peptone, whereas the level of lytic activity obtained in yeast extract was approximately three times that in peptone. Growth of the myxobacter in these media was characterized by a lag of 12 hr, followed by a rapid increase in cell population. After 36 hr, there was a dramatic decrease in turbidity of the cultures, resulting from massive cell lysis. Indeed, after 7 days of incubation, viable organisms could not be isolated from the media when streaked upon agar containing 1.0% yeast extract or cells of *A. crystallopoietes*.

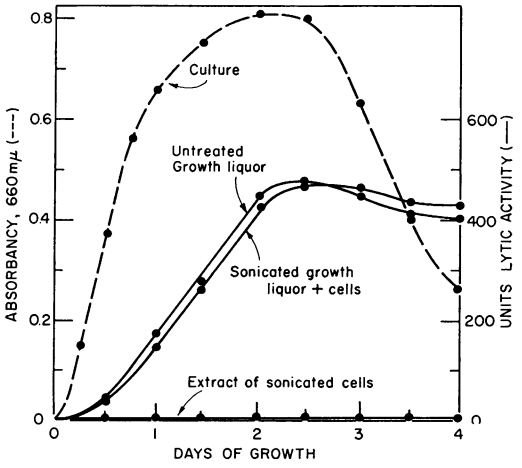

FIG. 2. *Location of the lytic enzyme at various stages of the growth cycle. Samples were removed from the growing culture at 12-hr intervals, and absorbancy was determined. Assays for the lytic enzyme were carried out with an untreated portion of the sample, a sonically treated portion of the sample, and sonically treated cells which had been removed from a separate portion of the sample.*

TABLE 2. *Effect of the purified enzyme on cells of myxobacter AL-1 harvested at different stages of growth*

Age of cells	Klett units decrease in 2 hr
hr	
12	0*
24	15
36	5
48	0
60	20
72	15
96	20

* Each value corrected for turbidity decrease of a control containing cells but no enzyme; in no instance did this value exceed 10 Klett units.

Approximately 80% of the lytic activity appeared during the second day of growth. The essentially constant level of activity maintained between 2 and 5 days indicates that the lytic factor is quite stable. Lytic activity was not lost upon dialysis of the yeast extract growth liquor for 48 hr at 4 C; it could be precipitated by ammonium sulfate and was destroyed by boiling.

An experiment was performed to determine whether the lytic enzyme was exo- or endocellular. A 1-liter amount of 1.0% yeast extract broth in a 3-liter Erlenmeyer flask was inoculated with 50 ml of a 24-hr broth culture and shaken for 4 days. At 12-hr intervals, a 50-ml sample was removed, the cells were sedimented by centrifugation, were washed once with 0.025 M Tris buffer at *p*H 9.0, and were suspended in this buffer at a final cell density of 600 Klett units; 3 ml of the cell suspension were placed in a lusteroid centrifuge tube (15 by 60 mm) stoppered with a parafilm-covered rubber stopper, and placed in the chamber of a sonic oscillator. This technique was used because of the small volume involved. As a control, 3 ml of the culture medium from which the cells had not been removed were similarly placed in the sonic oscillator. Water was added to the sonic oscillator cup to a level equal to that in the tubes. The organisms were then sonically treated for 10 min; virtually complete cell disruption resulted. The tubes were centrifuged at 15,000 × *g* for 20 min at 4 C, and the supernatant fluids were assayed for lytic activity. As shown in Fig. 2, no lytic activity was released from the disrupted cell suspensions. The same level of activity was maintained in the untreated growth liquor and in the sonically treated medium containing cells, showing again that enzyme was not released upon cell disruption and that the enzyme was stable to sonic treatment.

A sample of the washed myxobacter cells obtained at each time interval in the above experiment was tested for susceptibility to the purified enzyme (purification procedure presented below). To 2.0 ml of the cell suspension were added 2.5 ml of 0.025 M Tris buffer (*p*H 9.0) and 0.5 ml of enzyme solution. The tubes were incubated at 38 C for 2 hr while changes in optical density were followed. A tube without enzyme was likewise incubated as an autolysis control. As shown in Table 2, the organism proved resistant at all growth stages to the action of the enzyme.

The rate of lysis of suspensions of fresh cells, heat-killed cells, and cell walls of *A. crystallopoietes* by 0.2 ml of purified enzyme is illustrated in Fig. 3. The intact fresh cells essentially were lysed completely within 3 min. Heat-killed cells were lysed more slowly, requiring approximately 15 min for complete clearing. The cell-wall suspension became essentially clear after 25 min of digestion. The lysis of both heated and fresh cells was accompanied by a marked increase in viscosity, which was reduced completely within several minutes after the addition of deoxyribonuclease.

To test the possibility that the enzyme preparation contained proteolytic as well as cell-wall lytic activity, a 0.2% solution of bovine serum albumin was incubated with the enzyme, and the increase in the number of free amino groups was measured. The results (Fig. 4) show that there was a rapid release of DFNB reactive groups for 2 hr, followed by a slower rate of release, and finally reaching a maximal value after 10 hr of incubation. Both proteolytic and cell-wall lytic

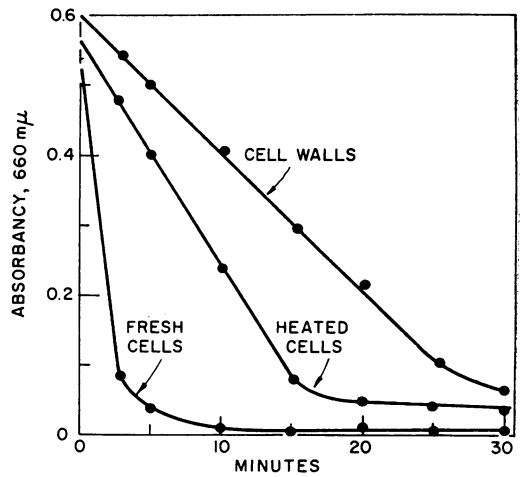

FIG. 3. *Rates of lysis of fresh cells, heat-killed cells, and cell walls of Arthrobacter crystallopoietes by purified enzyme. See Materials and Methods for assay techniques and Table 3 for details of enzyme purification.*

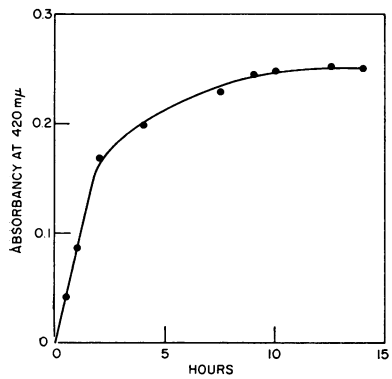

FIG. 4. *Release of dinitrofluorobenzene-reactive amino groups upon digestion of bovine serum albumin with purified enzyme. Values have been corrected for appropriate blanks.*

activities were precipitated from growth liquor by an appropriate concentration of $(NH_4)_2SO_4$ or acetone. The results of a purification procedure employing these two techniques as well as a negative diethylaminoethyl (DEAE) cellulose adsorption are presented in Table 3. At each step, assays for both cell-wall lysis and proteolysis were performed. To 8 liters of growth liquor at 4 C was added slowly and with vigorous stirring 0.75 volume (6 liters) of acetone precooled to −20 C. The turbid suspension was clarified by passage through a pad of Celite and Whatman no. 1 filter paper on a large Büchner funnel. The precipitate deposited on the pad was extracted with 500 ml of cold Tris buffer, but the clear, amber extract was devoid of enzymatic activity.

To the clear supernatant solution was added another 0.75 volume (6 liters) of cold acetone. Upon standing for 30 min, a flocculent precipitate formed and settled to the bottom of the vessel. The precipitate was collected on a Celite filter cake; the enzyme was extracted into two successive 500-ml portions of cold 0.05 M Tris buffer at *p*H 9.0, and residual acetone was removed from the combined supernatant fluids under vacuum. The acetone precipitation resulted in a 15.5-fold increase in specific activity for cell-wall lytic activity and a 14-fold increase in proteolytic activity, with an 87% recovery of both activities. The supernatant solution, after extensive dialysis to remove the acetone present, exhibited no enzymatic activity.

The increase in specific activity of cell-wall lysis obtained upon dialysis of the growth liquor is presumably a result of the removal of dialyzable materials which react with Folin's reagent, giving a false, high protein value. Proteolytic activity could not be measured in the undialyzed liquor, owing to a very high blank resulting from the dialyzable amino compounds.

The active fraction obtained from acetone treatment was precipitated by the stepwise addition of solid $(NH_4)_2SO_4$ with stirring at 4 C. The fraction obtained at 0 to 45% saturation contained some protein but no activity. The majority of both cell-wall lytic and proteolytic activity was obtained in a 45 to 65% precipitate. The precipitate was collected by centrifugation, was dissolved in 100 ml of 0.02 M Tris buffer at *p*H 9.0, and was dialyzed for 24 hr against several changes of this buffer. Approximately a twofold increase in the specific activities of both cell-wall lysis and proteolysis was obtained. At this stage, 72% of the cell-wall lytic and 70% of the proteolytic activity remained. A small and approximately proportional amount of both activities was precipitated in a 65 to 85% $(NH_4)_2SO_4$ fraction which, because of the low specific activity, was discarded.

To the dialyzed 45 to 65% fraction were added 10 g of DEAE-cellulose which had been equilibrated previously with 0.02 M Tris buffer at *p*H 9.0, and from which excess moisture had been removed on a Büchner funnel. The DEAE cellulose-enzyme suspension was stirred for 30 min at 4 C, followed by filtration through a Büchner funnel. The DEAE cellulose residue was washed with two successive 10-ml portions of Tris buffer at *p*H 9.0, and the filtrates were pooled. Approximately half of the protein in the preparation was taken up by the DEAE cellulose, whereas essentially none of the activity was adsorbed or lost. Thus, nearly a twofold increase in the specific activities of both cell-wall lysis and proteolysis resulted. A second, identical DEAE-cellulose treatment yielded only a negligible change in the specific activities.

This procedure resulted in a 60-fold purification of cell-wall lytic activity with a 69% recovery and a 57-fold purification of proteolytic activity with 70% recovery. These calculations have been made with the activity of dialyzed growth liquor used as initial values. Unfortunately, the purification of the enzyme was accompanied by the concentration of a highly viscous substance. This material, apparently a part of the slime which is readily observed when the organism is grown on solid media, prevented us from using column chromatography successfully.

The purified enzyme was tested for its ability to lyse fresh, unheated cells of a variety of microorganisms. Each organism was harvested during late-exponential or early-stationary phase, was washed once with 0.02 M Tris buffer at *p*H 9.0, and was suspended in this buffer at a cell density of from 450 to 550 Klett units. To 4.8 ml of cell

TABLE 3. *Purification of cell-wall lytic and proteolytic activities from growth liquor of myxobacter AL-1*

Fraction	Specific activity*		Total units		Per cent recovery	
	Cell-wall lysis	Proteolysis	Cell-wall lysis $\times 10^5$	Proteolysis $\times 10^4$	Cell-wall lysis	Proteolysis
Growth liquor	68	—	1.50	—	—	—
Dialyzed growth liquor	140	15	1.47	1.58	98.0	—
0 to 0.75 volume acetone precipitate	0	0	0.00	0.00	—	—
0.75 to 1.5 volume acetone precipitate	2,180	210	1.30	1.37	87.0	87.0
0 to 45% $(NH_4)_2SO_4$	0	0	0.00	0.00	—	—
45 to 65% $(NH_4)_2SO_4$	4,550	435	1.08	1.10	72.0	70.0
65 to 80% $(NH_4)_2SO_4$	1,580	135	0.17	0.14	11.3	14.5
First DEAE supernatant fraction	8,320	854	1.06	1.08	70.5	69.8
Second DEAE supernatant fraction	8,355	855	1.04	1.11	69.4	70.1

* Cell-wall lysis: decrease in optical density at 660 mμ of 0.001 in 15 min per mg of protein. Proteolysis: increase in optical density at 420 mμ of 0.001 in 1 hr per mg of protein.

TABLE 4. *Survey of the lytic activity of purified myxobacter enzyme against various microorganisms**

Organism	Per cent lysis
Lysis in 5 min	
Arthrobacter crystallopoietes	98
A. pascens	94
A. globiformis	95
Micrococcus lysodeikticus	92
Staphylococcus aureus	91
Rhodospirillum rubrum	99
Spirillum serpens	99
S. itersonii	98
Lysis in 15 min	
A. citreus	92
A. atrocyaneus	96
A. tumescens	91
Lysis in 1 hr	
A. ureafaciens	55
A. aurescens	70
Bacillus megaterium	44
B. thuringiensis	38
B. alvei	52
Streptococcus lactis	40
Sarcina lutea	27
A. oxydans	0
A. nicotiniae	0
Saccharomyces cerevisiae	0
Aerobacter aerogenes	0
Escherichia coli	0
Pseudomonas aeruginosa	0
P. fluorescens	0
Cell walls lysed in 15 min	
Arthrobacter crystallopoietes	86
Staphylococcus aureus	84
B. alvei	72
Euglena gracilis†	92

* We are grateful to the following individuals for supplying cultures of bacteria: R. Martinez, J. Hoch, and A. G. Lochhead.

† Prepared by R. H. Hurlbert.

suspension was added 0.2 ml of enzyme; the mixture was incubated at 38 C, and the turbidity was followed for 1 hr. Each suspension of cells or cell walls without enzyme was similarly assayed as an autolysis control; in no instance did the change exceed 0.01 absorbancy. The data presented in Table 4 include this correction. Of 10 species of *Arthrobacter* tested, 3 were lysed within 5 min, 3 within 15 min, 2 were lysed slowly, and 2 proved completely resistant to the enzyme. All of the gram-negative organisms tested were resistant to the enzyme, with the dramatic exceptions of *Rhodospirillum rubrum*, *Spirillum itersonii*, and *S. serpens*, which were lysed very rapidly. No pattern is evident with the gram-positive organisms, some being very susceptible and others less so; still others were completely resistant to the enzyme. The one yeast species tested was not lysed. Purified cell-wall suspensions of four organisms were completely cleared within 15 min. Cell walls of *Bacillus alvei* proved highly susceptible to the enzyme, whereas fresh intact cells were only slowly lysed.

DISCUSSION

The properties of creeping motility, cell flexibility, and copious slime production indicate that the bacteriolytic organism is a member of the order Myxobacterales as defined in *Bergey's Manual*. The establishment within this order of the family Cytophagaceae is based upon the failure of these myxobacteria to produce fruiting bodies or microcysts. Using this classification, our organism should be considered as a member of the genus *Cytophaga*. The eight fresh-water species of *Cytophaga* are characterized by growth upon cellulose, starch, or chitin. Our isolate grows upon none of these substrates. On the other hand, bacteriolysis has not been ascribed to this genus. Salton (1955) reported that *C.*

johnsonae lysed a species of yeast but exhibited no lysis upon bacterial cell walls. Proteolytic activity has been reported for certain species of *Cytophaga*. Stanier (1947) noted that *C. johnsonae* hydrolyzed gelatin and casein. McDonald, Quadling, and Chambers (1963) reported that an unidentified cold-tolerant species of *Cytophaga* exhibited proteolytic activity. The ability of many members of the order Myxobacterales to lyse cells and grow at the expense of other bacteria is well known. We prefer to designate the organism as myxobacter strain AL-1, since it is not readily classified by present schemes. An example of a similar problem is the variant of *Myxococcus xanthus* described by Dworkin (1962); this variant forms no fruiting bodies or microcysts and might be classified as a species of *Cytophaga* were its origin unknown.

The origin of an enzyme in the growth medium must be interpreted with caution. As emphasized by Pollock (1962), the most reliable criterion for determining the origin of an enzyme is to compare its concentration inside (or bound to) the cell with that in the medium. The absence of lytic activity in sonically disrupted cells of the myxobacter at all stages of the growth cycle is supporting evidence that the enzyme is exocellular. A comparison of the levels of lytic activity found in sonically treated growth liquor containing cells with that in the untreated supernatant liquid indicates that the enzyme was not altered by the sonic treatment and was not bound to the cell surface.

Bacteriolytic enzymes have been isolated from cultures of a wide variety of microorganisms, including species of *Streptomyces* (Salton, 1955; Ghuysen, 1957), species of *Myxococcus* (Bender, 1962; Norén, 1960), *B. subtilis* (Richmond, 1959), *Flavobacterium* (Kato et al., 1962), *Staphylococcus aureus* (Schindler and Schuhardt, 1964), and the fungus, *Chalaropsis* sp. (Hash, 1963). Each of these enzymes attacks gram-positive bacteria, the types varying widely with each enzyme. None of these enzymes has been shown to lyse gram-negative bacteria which had not been treated previously with agents such as ethylenediaminetetraacetate (EDTA) and butanol. The enzyme from myxobacter AL-1 likewise exhibits specificity as to the gram-positive bacteria attacked, some being highly susceptible, others less so, and still others completely resistant. With three exceptions, the myxobacter enzyme, like the other bacteriolysins, does not attack intact gram-negative bacteria. The exceptions, *R. rubrum*, *S. serpens*, and *S. itersonii* were lysed almost instantaneously by the purified enzyme. It perhaps is significant that two of these organisms, *S. serpens* (Murray, 1963) and *R. rubrum* (Salton and Williams, 1954), possess a macromolecular outer layer.

The observation that organisms of the genus *Arthrobacter* vary widely in their susceptibility to the lytic enzyme was surprising. Cummins and Harris (1959) reported that five species of *Arthrobacter* contain lysine, whereas two others contain diaminopimelic acid (DAP), as a component of their cell walls. There is apparently no relation between these two groups and susceptibility to the myxobacter enzyme, because *A. citreus*, containing lysine, and *A. tumescens*, containing DAP, were lysed at essentially the same rate. Also *A. globiformis* and *A. aurescens*, both containing lysine, were lysed at widely differing rates.

One enzyme may be responsible for both cell-wall lysis and proteolysis, since purification procedures produced an essentially parallel increase in specific activity at each step of purification. Studies of this enzyme are being continued.

Gillespie and Cook (1965) recently reported on the lytic enzymes of strains of sorangia. Since *Sorangium* is characterized by the production of fruiting bodies and microcysts, these organisms appear unrelated to the myxobacter reported here.

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