THE EXOCELLULAR BACTERIOLYTIC SYSTEM OF SOIL ACTINOMYCES

II. A FURTHER INVESTIGATION OF THE LYTIC SYSTEM

by

P. W. MUGGLETON AND M. WEBB

Chemistry Department, University of Birmingham (England)

The preliminary findings of an investigation of the nature and properties of the exocellular bacteriolytic system of a soil *Actinomyces* Sp. were recorded in a previous paper (JONES, SWALLOW AND WEBB[1]). It was then shown, in agreement with the work of WELSCH[2,3], that the lytic system contained a bactericidal substance, probably of a lipoid nature, and a proteolytic enzyme system. The bactericidal substance was active against Gram positive, but inactive against Gram negative bacteria. This finding was consistent with the fact that the system lysed-killed Gram negative organisms, but had no action against them when they were living. In the presence of the bactericidal substance, Gram positive cells were killed and subsequently became Gram negative; in this state they were susceptible to lysis by the proteolytic enzyme system. The lysis of killed Gram positive cells by filtrates from cultures of the *Actinomyces* Sp. in complex media was associated with the presence of a ribonuclease. Synthetic medium culture filtrates which were examined at the time had no ribonuclease activity and were unable to bring about the lysis of heat-killed Gram positive cells unless the latter were first rendered Gram negative by pancreatic ribonuclease. It was concluded, therefore, that the proteolytic enzyme system could not lyse the cells whilst the Gram complex remained intact.

WELSCH[4] has shown that the lytic principle from his *Actinomycete* can lyse living suspensions of *Staphylococcus aureus*, and *B. megatherium* and has claimed that this action is not due to a bactericidal agent which kills the cells under conditions which permit the action of their autolytic enzymes. In a subsequent paper (WELSCH[5]), it was shown that concentrates of the lytic principle after extraction of the lipoids could also bring about the lysis of living Gram positive cells. Since it was not possible to demonstrate ribonuclease activity in these preparations, WELSCH suggested that the cells need not necessarily be rendered Gram negative before lysis occurs. With the *Actinomyces* Sp. used in these studies, however, the lytic activity of culture filtrates against Gram positive organisms is invariably associated with the presence of a ribonuclease. Indeed subsequent studies have shown that under modified cultural conditions, culture filtrates from the *Actinomyces* (strain "A") in synthetic as well as in complex media, are able to lyse heat-killed Gram positive cells and contain an enzyme of the ribonuclease type.

References p. 441.

The proteolytic enzyme system of the *Actinomyces "A"* was shown in Part I of this series (JONES, SWALLOW AND WEBB[1]) to consist of at least two enzymes. One of these, a proteinase, hydrolysed proteins of high molecular weight and the other, a peptidase, hydrolysed peptides (*e.g.* Witte peptone). It was suggested that the lysis of Gram negative cells resulted from the action of the proteinase on the cell proteins, and that further hydrolysis of the split products was brought about by the peptidase.

The results of a further investigation of the enzymes of the bacteriolytic system of an *Actinomyces* Sp. are described in the present communication. Studies of the nature and characterisation of these enzymes are recorded in the following paper (Part 3).

EXPERIMENTAL

Growth of the Actinomyces

The strain of soil *Actinomyces* referred to in Part I as *Actinomyces* "A" was used throughout the present study. It was maintained in Lemco-peptone broth and subcultured at monthly intervals. For the production of the enzyme system, the strain was grown in mould culture flasks containing 500 ml of the chemically-defined medium ("mannitol-synthetic-soil"). After 3–4 weeks' growth at 25°, the organism formed a confluent, crumbly, surface growth of a light brown colour with a few small, discrete colonies at the bottom of the medium. Some spore formation commenced after about 10 days. Filtration through coarse filter paper yielded a water-clear, pale brown, filtrate.

Estimation of bacterial cell lysis

Except where otherwise stated, bacteriolytic activity was determined against killed *Bact. lactis aerogenes* cells. The bacterial cells were heat-killed by the method of DUBOS AND MACLEOD[6] and were suspended in 0.85% sodium chloride to give a suspension of opacity equivalent to 5 times that of the tube No. 10 of MacFarlane's $BaSO_4$ standard. For each determination a series of 6 tubes was used into which were placed 1.0, 0.75, 0.5, 0.25, 0.1 and 0.0 ml of the enzyme solution respectively. 0.1 *M* phosphate buffer pH 7.0 (1.0 ml), 1/1000 merthiolate (sodium ethyl mercurithiosalicylate, 0.25 ml) and the cell suspension (1.0 ml) were then added to each tube, and the volumes adjusted to 5 ml with 0.85% sodium chloride. After 48 hrs at 37°, the opacity of each suspension was estimated turbidimetrically with the Spekker photoelectric absorptiometer.

Experiments in which the enzyme-substrate system was overlaid with vaseline, or kept under hydrogen after first removing dissolved air by boiling *in vacuo*, showed that anaerobic conditions had no effect upon the rate of lysis.

Estimation of proteinase activity

Proteinase activity was measured by the decrease in viscosity of a protein (*i.e.* casein) substrate (NORTHROP AND KUNITZ[7], LENNOX[8]). Activity determinations were made initially in viscometers of the "totally enclosed" type (KOCH, ORTHMAN AND DEGENFELDER[9]), later determinations were carried out in Ostwald viscometers at 37°.

3 ml of a 10% solution of "light white" casein in 0.1 *M* phosphate buffer, pH 7.0, were introduced into the viscometer followed by 1 ml of 0.85% sodium chloride. After temperature equilibration, 2 ml of a suitable dilution of the enzyme solution, preheated to 37° were added. Measurements of viscosity (Flow time) were made initially and every 2 minutes for the following 10 minutes.

The proteinase activity was expressed in arbitrary units such that, under the above conditions, an activity of 100 units/ml resulted in a 5% reduction in flow time of the enzyme-substrate system in 10 minutes. It was found experimentally that enzyme activity up to about 250 U/ml was directly proportional to per cent reduction of flow time.

Estimation of peptidase activity

Peptidase activity was measured by the formol titration using a peptone substrate as previously described (JONES *et al.*[1]). The accuracy of the estimations was increased by following the titration potentiometrically using the glass electrode according to the method developed by DUNN AND LOSHAKOFF[10] and applied to complex peptide substrates by FRENCH AND EDSALL[11]. In an attempt to obtain a substrate of uniform molecular size, Evans' peptone was fractionated according to the method of LANDSTEINER[12] for the separation of peptides containing 6–8 amino acids from silk hydrolysate. Under these conditions, however, only 1.52 g of product were obtained from 700 g of peptone. A suitable substrate was prepared by dialysis of a solution of the peptone against distilled water at 4°. Estimation of the α-amino nitrogen of the dialysate by the method of POPE AND STEVENS[13]

References p. 441.

before and after hydrolysis (36 hours) with 5 *N* hydrochloric acid, showed the molecules to average tri- or tetra-peptides. As the preparation of the peptidase substrate by dialysis of Evans' peptone was unlikely to be reproducible and since different peptides would be hydrolysed by the peptidase at different rates, the substrate was prepared in large quantity, concentrated, and stored under toluene at 4°. This preparation was used throughout the following experiments.

Determination of peptidase activity was carried out as follows: 5 ml of peptone solution (initial α-amino nitrogen content 0.656 mg/ml) was adjusted to pH 7.0 with sodium hydroxide, diluted with 0.85% sodium chloride (2.5 ml) and added to the enzyme solution (2.5 ml). Toluene (0.05 ml) was added and the mixture was incubated at 37°. Aliquots (1 ml) of the solution were withdrawn initially and at suitable intervals during the hydrolysis, added to 40% formaldehyde (1 ml) and water (3 ml) and titrated with 0.01 *N* sodium hydroxide in an atmosphere of nitrogen.

An arbitrary scale of peptidase activity was defined such that an enzyme solution containing 100 units of activity/ml gave an increase in titration of 1.0 ml of 0.01 *N* NaOH in 24 hours under the above conditions. The substrate concentration used was that which gave the maximum increase in titration with a constant enzyme concentration (c. 100 units/ml). With enzyme solutions ranging in activity from 10 to 200 units/ml, it was found that activity was almost proportional to the increase in titration after 24 hours.

The effect of time of incubation of the actinomyces cultures on the proteolytic enzyme production

Table I shows the proteinase and peptidase activities determined at weekly intervals in unshaken cultures incubated at 25°.

TABLE I

THE EFFECT OF TIME OF INCUBATION ON THE PROTEOLYTIC ENZYME PRODUCTION

Age of culture (weeks)	Activity (U/ml)		Lysis of killed cells (ml enzyme/5 ml for 30% lysis)
	Proteinase	Peptidase	
1	26	30	Over 1.0
2	45	36	0.46
3	113	48	0.15
4	100	52	0.09
5	142	42	0.13
6	138	73	0.09
7	123	43	0.10
8	71	55	0.20

The increase in bacteriolytic activity with increasing age of the culture to maximum activity at 4–5 weeks, is in agreement with previous findings and closely parallels the proteinase activity of the filtrates. As a result of these experiments, cultures used for the isolation of the enzymes were incubated for 4–5 weeks.

Concentration of the bacteriolytic enzyme system

In Part I it was shown that the bacteriolytic enzymes could be purified and concentrated by saturation of the culture filtrate with ammonium sulphate. This method was accompanied by a variable loss in enzyme activity. Loss of activity occurred also when the enzymes were precipitated or reprecipitated with ammonium sulphate. For example, a sample of culture filtrate lost 32.2% and 32.7% of its proteinase and peptidase activities respectively at the first precipitation and 48.5 and 33.8% respectively on reprecipitation with ammonium sulphate. From these figures it is concluded that the initial loss in activity does not represent the inactivation of a more labile enzyme.

Precipitation with acetone at −10°, although more successful, failed to give a

References p. 441.

sharp separation of the enzymes (*cf.* our forthcoming paper). Thus the activities were distributed amongst all the precipitates collected between acetone concentrations of 40 and 80%.

Both of the above methods were obviously unsuitable for the isolation of the enzymes from large volumes of culture filtrate and other methods for the isolation of the enzymes were studied.

Attempts to separate the enzymes by adsorption on $C\gamma$ alumina, kieselguhr, fuller's earth, bentonite and filter cel were unsuccessful.

Concentration of the enzymes was obtained, however, by dialysis and pervaporation.

The culture filtrate was dialysed against running tap water for 48 hours in cellophane tubes and then against distilled water for 24 hours. The tubes containing the dialysed solution were then hung in a fume chamber and the water removed by pervaporation. Using tubes 1½ inches in diameter, 300 ml of dialysed filtrate were concentrated to 40 ml in 48 hours.

During the process, a certain amount of insoluble, pale straw-coloured protein material (ash content = 6%) separated. This was sparingly soluble in water and contained about 35% of the total bacteriolytic activity of the culture filtrate. The formation of an insoluble precipitate occurred also when the culture filtrate was concentrated under reduced pressure at 30°. Further precipitation did not occur, however, when the concentrated (pervaporated) enzyme solution was diluted with water and again concentrated by pervaporation. Thus it was concluded that the insoluble material obtained during the initial concentration probably consisted of inert denatured protein, onto which the lytic enzymes were partially adsorbed, rather than the denatured enzymes themselves.

The concentrated (pervaporated) enzyme solution was dried in the frozen state to yield a pale straw-coloured, highly deliquescent solid which was stored at 4°. 1 Litre of filtrate from 4 week cultures yielded 0.28 g of solid. The material was redissolved in distilled water as required.

The losses of enzyme activity which occurred during the concentration of a batch of culture filtrate by this process are recorded in Table II.

TABLE II

LOSS OF ENZYME ACTIVITY ON CONCENTRATION OF THE CRUDE CULTURE FILTRATE

Process	Percentage loss of activity	
	Proteinase	Peptidase
Dialysis	40.6	53.5
Pervaporation	33.8	75.4
Freeze-drying	0	13.4

The loss of enzyme activity which occurred on dialysis (Table II) was probably due to the concurrent drop in p_H. The latter was attributed to the relatively slow diffusion of the large amount of sulphate ion present in the medium. In support of this it was found that 70% of both the proteinase and peptidase activities of the culture filtrate were lost when the solution was kept for 24 hours at p_H 5.0 and 4°, whereas a second dialysis of the concentrated, once dialysed (*i.e.* ion-free) solution, resulted in no further significant loss in activity.

References p. 441.

It was shown also that the enzymes slowly diffused through the cellophane membranes during dialysis, although the activity lost in this way during 48 hours was small.

Properties of the enzyme system

Optimum p_H for the lysis of heat-killed Gram negative cells

The optimum lytic activity of the system towards heat-killed cells of *Bact. lactis aerogenes* was shown in Part I to occur between p_H 7.0 and p_H 7.5. This value was confirmed in an experiment with cells of the same strain in which veronal hydrochloric acid buffers were used to cover the p_H range from 3.5 to 9.5. In view of this result, all subsequent experiments were carried out at p_H 7.0.

Inhibition of the lytic process by thiol compounds

The inhibitory action of hydrogen sulphide on the bacteriolytic activity of the Actinomyces enzyme system was recorded in the previous paper. Further experiments (Fig. 1) showed that the bacteriolytic activity was inhibited by glutathione or by cysteine, but not by methionine. In these experiments toluene was used as a preservative since reaction occurred between the sulphur-containing compounds and the merthiolate normally used.

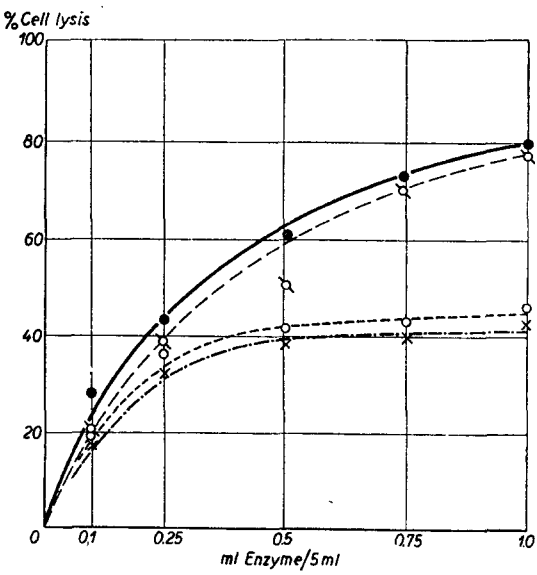

Fig. 1. The effect of methionine, glutathione and cysteine HCl on the lysis of heat-killed *Bact. lactis aerogenes* by Actinomyces bacteriolytic enzymes.

●———● Control
O- - - - - O 0.05 *M* glutathione
×—·—·—× 0.05 *M* cysteine HCl
O— — — O 0.05 *M* methionine

The role of the proteinase in the lysis of killed Gram negative cells

It was suggested in Part I that the lysis of killed Gram negative cells by the Actinomyces bacteriolytic enzyme system resulted from the action of the proteinase and that the peptides thus liberated were degraded further by the peptidase. Proof of this hypothesis was obtained from a study of the action of the proteinase alone on killed Gram negative cells. The proteinase was obtained free from peptidase activity by preferential deactivation of the latter at 60° according to the method described in the following paper. Thus a sample of filtrate, having initially a proteinase activity of 122 U/ml and a peptidase activity of 82 U/ml, after 45 minutes at 60° had 66 U/ml of proteinase and no peptidase activity. The lytic activity of this solution against heat-killed cells of *Bact. lactis aerogenes* was compared with a solution of the unheated filtrate diluted with 0.85% sodium chloride. The latter solution contained the same proteinase activity (66 U/ml) as the heated solution, but in addition contained 44 U/ml of the peptidase.

References p. 441.

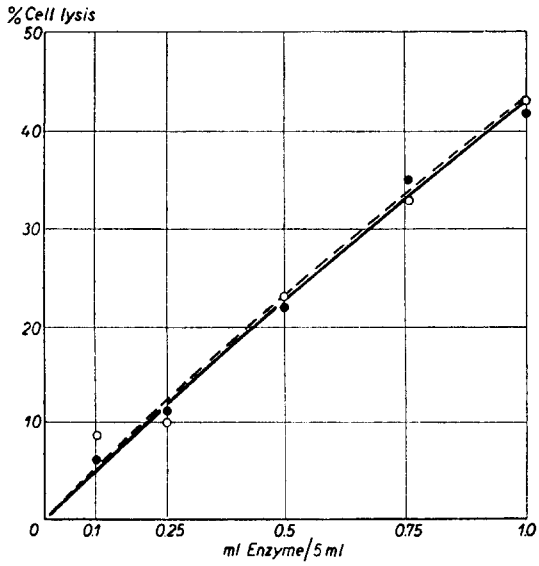

Fig. 2. The lysis of heat-killed *Bact. lactis aerogenes* by an Actinomyces culture filtrate in which the peptidase is inactivated by heat.

●——● Unheated (Diluted) Proteinase 66 U/ml Peptidase 44 U/ml

○— — —○ Heated Proteinase 66 U/ml Peptidase 0 U/ml

The results (Fig. 2) show that the presence of the peptidase has no influence upon the lysis of the cells.

Adsorption of the proteolytic enzymes on heat-killed Gram negative cells

A suspension of killed *Bact. lactis aerogenes* cells (10 ml) equal in opacity to 5 times tube 10 of the Mac Farlane standard, was added to a mixture of the purified enzyme solution (10 ml) and 0.1 *M* phosphate buffer p_H 7.0 (10 ml). The suspension was incubated at 37° in the presence of toluene (0.5 ml). Samples of the suspension were withdrawn at 24 hour intervals and the cells removed in the centrifuge. The lytic activities of the clear supernatants were measured against heat-killed *Bact. lactis aerogenes* cells. The per cent loss of activity (Table III), based on the activity of the 0-hour sample was calculated by reading from the activity curves the amount of supernatant required to accomplish 30% lysis in 24 hours. In the control experiment the cell suspension was replaced with 0.85% sodium chloride (10 ml).

It is evident from the results (Table III) that during lysis the lytic enzymes are taken up by the cells.

TABLE III

ADSORPTION OF THE BACTERIOLYTIC SYSTEM ON HEAT-KILLED *Bact. lactis aerogenes* CELLS

Time (hours)	Percent. loss of lytic activity of the Actinomyces enzyme solution	
	Enzyme solution incubated with cells	Control (enzyme solution incubated alone)
0	0	0
24	39	23
48	60	32
72	73	37

In an extension of the work outlined above, the proteinase and peptidase activities of the supernatant solutions were measured at intervals after removal of the cells. Cell lysis was measured at the same time using the Spekker absorptiometer. The results (Fig. 3) were confirmed by a number of similar experiments, and show that when the enzyme solution is incubated with heat-killed Gram negative cells, the proteinase is rapidly adsorbed. Adsorption of the proteinase was followed by the lysis of the cells.

References p. 441.

The latter was accompanied by the liberation of the products of proteolysis, as evidenced by an increase in the α-amino- and total nitrogen of the supernatant solution. These split products formed a substrate for the peptidase, which, after an initial time lag, was partially removed from solution.

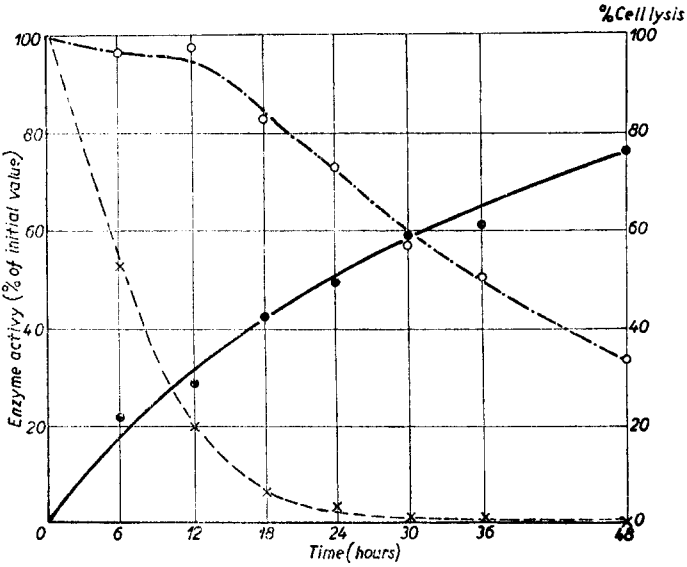

Fig. 3. Adsorption of the proteolytic enzymes of the Actinomyces culture filtrate by heat-killed *Bact. lactis aerogenes* cells.

●———● Cell lysis; ×— — —× Proteinase in supernatant; O-·—·-O Peptidase in supernatant

Lysis of Gram positive cells by the Actinomyces lytic enzyme system

In Part I it was shown that culture filtrates of the Actinomyces strain grown in a complex medium were able to lyse suspensions of heat-killed Gram positive bacteria. This property was associated with the presence in the filtrate of an enzyme of the ribonuclease type which rendered the cells Gram negative and therefore susceptible to lysis by the proteolytic enzyme system.

Subsequently it has been found that cultures grown in the synthetic medium also produce a ribonuclease, and that filtrates from these cultures lyse both killed Gram positive and Gram negative organisms.

The lysis of various heat-killed Gram positive organisms by the bacteriolytic enzymes isolated from cultures of *Actinomyces* "A" in the synthetic medium is shown in Fig. 4. For these determinations, *Staphylococcus aureus* was grown on nutrient agar, whilst *Corynebacterium diphtheriae, Streptococcus haemolyticus* and *Lactobacillus delbruckii* strains were grown on nutrient agar containing 5% horse serum. The *Clostridium welchii* cells were grown in 0.2% glucose broth under anaerobic conditions.

Stained films prepared from the heat-killed bacteria after 12 hours contact with the enzyme showed that the cells were first rendered Gram negative before they were lysed by the proteolytic enzymes. Thus in the highest concentration of the enzyme (1.0 ml/5 ml) the cells were about 85% Gram negative as compared with 5% Gram negatives in the control. The ribonuclease activity of the enzyme solution was determined by following the hydrolysis of a 0.1% solution of sodium ribonucleate at p_H 7.0

References p. 441.

by the method of DAVIDSON AND WAYMOUTH[14], 90% hydrolysis to acid soluble nucleotides was found in 7 hours at 37°.

It was observed that the lysis of suspensions of heat-killed Gram positive cells by the Actinomyces enzyme system occurred more slowly than did the lysis of heat-killed Gram negative cells. Thus it appeared possible that the conversion of the killed Gram positive cells to the Gram negative state was the rate determining factor. Accordingly, the lysis of heat-killed cells of *Bact. lactis aerogenes*, of heat-killed *Cl. welchii* cells and of *Cl. welchii* cells rendered Gram negative by extraction with sodium cholate (HENRY AND STACEY[15]) and by autolysis, were compared.

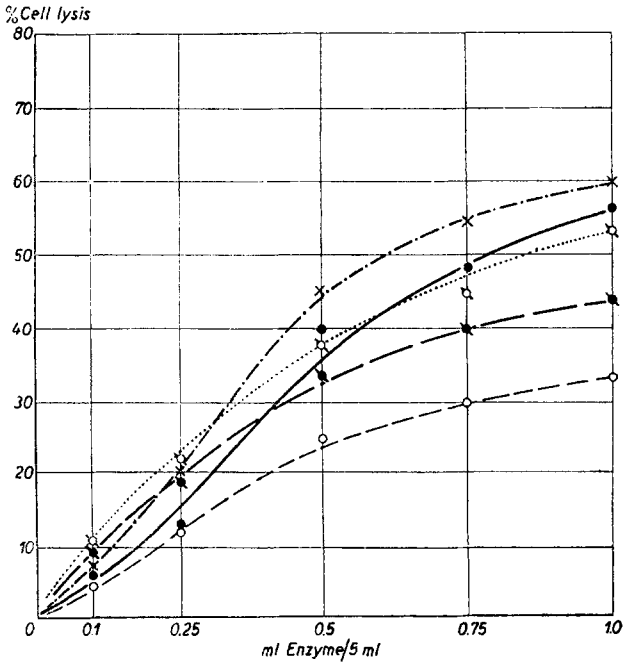

Fig. 4. The lysis of heat-killed Gram positive cells by the Actinomyces enzymes in a synthetic medium culture filtrate

●———● *Streptococcus haemolyticus* 618 O— — —O *Staphylococcus aureus* "A"
×–·—·—·–× *Corynebacterium diphtheriae* O------O *Lactobacillus delbruckii*
●— — —● *Clostridium welchii*

The cells from a 14 hour anaerobic culture of *Cl. welchii* in Lemco broth containing 0.2% glucose were collected and washed in 0.85% sodium chloride (Sharples centrifuge). The cells were suspended in 0.85% sodium chloride and the suspension divided into three parts. The cells of one part of the suspension were heat-killed in the usual manner. The second fraction of the cells was extracted with 2% sodium cholate at 60° until completely Gram negative (48 hours) and then washed at the centrifuge with 0.85% sodium chloride until free from sodium cholate. The third part was allowed to autolyse at 37° at p_H 7.0 until all the cells just became Gram negative (6 hours) (*cf.* HENRY, STACEY AND TEECE[16]). They were washed in 0.85% sodium chloride and then maintained at 80° for 2 hours.

The lytic activity of a solution of purified Actinomyces enzyme against the three

References p. 441.

suspensions of *Cl. welchii* cells and a suspension of heat-killed *Bact. lactis aerogenes* was measured in the usual way. For comparison, the lysis of the same 4 suspensions by a solution of commercial trypsin (which contained a weak ribonuclease activity) diluted to give the same casein splitting activity as the Actinomyces solution (175 U/ml) was determined.

From the results (Fig. 5 and 6), it is apparent that the Gram negative forms of *Cl. welchii* are lysed considerably more rapidly than are the killed Gram positive *Cl. welchii* cells. This marked difference in the rates of lysis may be attributed to the inability of the proteolytic enzymes to attack the Gram positive cells before they have been rendered Gram negative by the ribonuclease.

The slightly greater lysis of the *Cl. welchii* cells which were rendered Gram negative by partial autolysis is attributed to the fact that during the autolysis some degradation of the cell proteins occurred in addition to the destruction of the Gram complex. The extent of the lysis of either *Bact. lactis aerogenes* or autolysed *Cl. welchii* cells by trypsin was equal to that due to the Actinomyces enzyme. Furthermore the fact that little lysis of the killed Gram positive *Cl. welchii* cells occurred as a result of the action of the trypsin is consistent with the presence of the weak ribonuclease activity in the enzyme preparation and shows that killed Gram positive cells are resistant to the action of proteinases of the trypsin type.

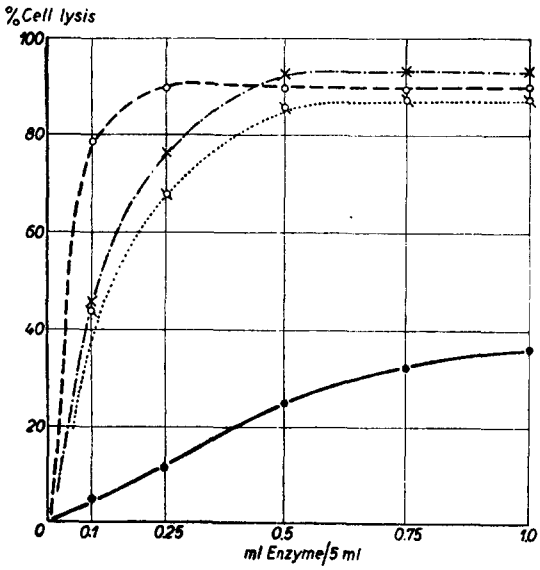

Fig. 5. The lysis of Gram negative forms of *Clostridium welchii* by the Actinomyces enzyme system.

- ●———● *Cl. welchii* cells. Heat-killed (Gram positive)
- O— — —O *Cl. welchii* cells, rendered Gram negative by autolysis
- ×—·—·—× *Cl. welchii* cells, rendered Gram negative by cholate extraction
- O------O *Bact. lactis aerogenes* cells. Heat-killed

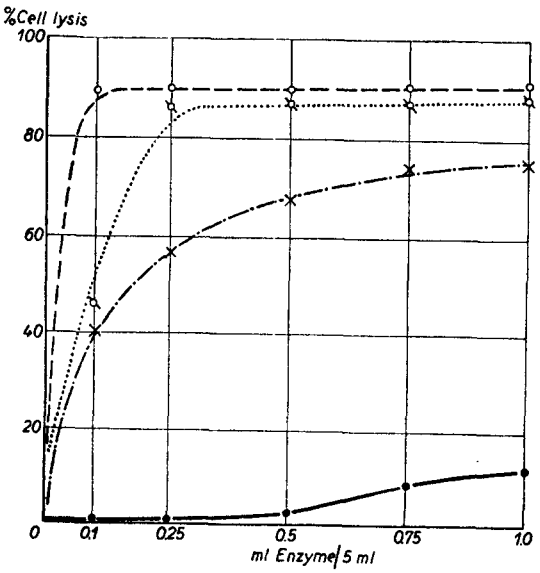

Fig. 6. The lysis of Gram negative forms of *Clostridium welchii* by trypsin.

- ●———● *Cl. welchii* cells. Heat-killed (Gram positive)
- O— — —O *Cl. welchii* cells, rendered Gram negative by autolysis
- ×—·—·—× *Cl. welchii* cells, rendered Gram negative by cholate extraction
- ●------● *Bact. lactis aerogenes* cells. Heat-killed

References p. 441.

DISCUSSION

In the foregoing experimental it has been shown that the lysis of a suspension of heat-killed Gram negative bacterial cells by the Actinomyces bacteriolytic system is brought about by a proteinase which hydrolyses high molecular weight proteins. Before lysis commences the enzyme is adsorbed onto the cells. As lysis proceeds the cell proteins are broken down and the resulting products in turn provide a substrate for the peptidase.

The fact that the proteinase is similar in its action to trypsin is evident from the finding that both enzymes when diluted to the same casein hydrolysing activity, lyse-killed Gram negative cells and Gram positive cells rendered Gram negative by autolysis at the same rate. Furthermore the Actinomyces proteinase is similar to trypsin in so far as its action is inhibited by thiol compounds.

No indication has been obtained that the Actinomyces proteolytic enzyme system itself is able to lyse heat-killed Gram positive cells until these have been rendered Gram negative. The lytic activity of culture filtrates against killed Gram positive cells has been shown to be dependent upon the presence in the filtrate of an enzyme of the ribonuclease type. This enzyme, which renders the killed Gram positive cells Gram negative and thereby susceptible to lysis by the proteolytic enzyme system, has been the subject of a detailed investigation, the results of which will be presented in Part 4 of this series.

SUMMARY

The bacteriolytic action of culture filtrates of a strain of soil actinomyces against killed Gram negative cells is shown to be due to the action of the proteinase component of a proteolytic enzyme system. The bacteriolytic action of the proteinase is optimal at p_H 7, and is inhibited by thiol compounds.

The Actinomyces culture filtrates also contain an enzyme of the ribonuclease type. Killed Gram positive cells are rendered Gram negative by the action of the ribonuclease and in this state are susceptible to lysis by the proteinase.

The concentration and partial purification of the enzymes of the Actinomyces bacteriolytic system by a method involving dialysis, pervaporation and freeze-drying is described.

RÉSUMÉ

Il est montré dans ce travail que l'action bactériolytique d'un filtrat de culture d'une souche d'Actinomyces sur des cellules Gram négatives tuées est due a l'action d'un composant de la protéinase d'un système d'enzyme protéolytique. L'action bactériolytique de la proteinase est optima pour $p_H = 7$ et est inhibée par les composes thiols.

Les filtrats de culture d'Actinomyces contiennent aussi une enzyme du type ribonuclease. Les cellules Gram positives tuees sont rendues Gram négatives par l'action de la ribonuclease et sous cet état sont susceptibles d'etre lysées par la proteinase.

Une méthode pour la concentration et la purification partielle des enzymes du système bactériolytique des Actinomyces consistant en dialyse, pervaporation et dessication a froid est egalement décrite.

ZUSAMMENFASSUNG

Der bakteriolytische Effekt von Kulturfiltraten eines Stammes von Erde-Aktinomyces auf abgetötete Gram-negative Zellen kann auf die Wirkung der Proteinase des proteolytischen Enzymsystems zurückgeführt werden. Die bakteriolytische Wirkung der Proteinase hat ihr Optimum bei p_H 7 und wird durch Thiol-Verbindungen gehemmt.

Das Filtrat der Aktinomyces Kulturen enthält auch eine Ribonuklease, die abgetötete Gram-positive Bakterien Gram-negativ macht und so für die Lösung durch die Proteinase vorbereitet.

References p. 441.

Die Konzentrierung und Teilreinigung der Enzyme des bakteriolytischen Systems des Aktinomyces durch Dialyse, Pervaporation und Trocken-Frierung wird beschrieben.

REFERENCES

[1] A. S. JONES, A. J. SWALLOW, AND M. WEBB, *Biochim. Biophys. Acta*, 2 (1948) 167.
[2] M. WELSCH, *J. Bact.*, 42 (1941) 801.
[3] M. WELSCH, *J. Bact.*, 44 (1942) 571.
[4] M. WELSCH, *Bull. soc. chim. biol.*, 29 (1947) 362.
[5] M. WELSCH, *Compt. rend. soc. biol.*, 142 (1948) 1589.
[6] R. J. DUBOS AND C. M. MACLEOD, *J. Exptl Med.*, 67 (1938) 791.
[7] J. H. NORTHROP AND M. KUNITZ, *J. Gen. Physiol.*, 16 (1932) 313.
[8] F. G. LENNOX, *J. Council Sci. Ind. Research*, 16 (1943) 155.
[9] J. R. KOCH, A. C. ORTHMAN, AND M. A. DEGENFELDER, *J. Am. Leather Chem. Assoc.*, 34 (1939) 489.
[10] M. S. DUNN AND F. A. LOSHAKOFF, *J. Biol. Chem.*, 113 (1936) 359.
[11] D. FRENCH AND J. T. EDSALL, *Advances in Protein Chem.*, 2 (1945) 277.
[12] K. LANDSTEINER, *J. Exptl Med.*, 75 (1942) 269.
[13] C. G. POPE AND M. F. STEVENS, *Biochem. J.*, 33 (1939) 1070.
[14] J. N. DAVIDSON AND C. WAYMOUTH, *Biochem. J.*, 38 (1944) 375.
[15] H. HENRY AND M. STACEY, *Proc. Roy. Soc.*, 133 (1946) 391.
[16] H. HENRY, M. STACEY, AND E. G. TEECE, *Nature*, 156 (1945) 720.

Received April 16th, 1951