N. J. Microbiology, 2(2), 1982, pp 154 – 167

POLYGALACTUROMASE AND PECTIN LYASE OF *Penicillium sclerotigenum*

By:

PATRICK O' OLUTIOLA

Department of Microbiology,
University of Ife, Ile-Ife,
Nigeria.

Running Title

POLYGALACTURONASE AND PECTIN LYASE FROM PENICILIUM

ABSTRACT

Penicillium sclerotigenum Yamamoto grew in synthetic liquid medium with pectic substance as sole carbon source, releasing proteins which exhibited polygalacturonase (PG; poly (1,4-&-D-gal-acturonide) glycohydrolase, EC 32.1.15) and pectin lyase (PL; poly (methoxygalaturonide) lyase, EC 4.2.2.10) activitie . The enzymes were also present in mycelial extracts and *Pencillium*-infected yam tissues but were absent in healthy yam tissues. These enzymes were separated by molecular exclusion and ion exchange chromatography into three components. The molecular weight, estimated by gel filtration on sephadex G-100, was approximately 72,000 Optimum temperature for activity of polygalacturonase and pectin lyase was 35°C. The pH optima for polygalacturonase and pectin lyase were pH 5.0 and pH 8.5 respectively.

INTRODUCTION

Many pathogenic microorganisms have been reported to produce enzymes capable of degrading pectic substances in host tissues (Wood, 1960; Lund and Brocklehurst, 1978). Polygalacturonase (PG; poly (1, 4-& -D-galacturonide) glycohydrolase, EC 3.2.1.15) and pectin lyase (PL; poly (methoxygalacturonide) Iyase, EC 4.2.2.10) cause plant cell maceration and death of plant tissues (Garibaldi and Bateman, 1971; Mount, Bateman and Basham, 1970). Such enzymes will be of great importance where pathogens invade host plants in an intercellular manner. Hsu and Vaughn (1969) and Garibaldi and Bateman (1971) described pectic enzymes from pathogenic bacteria capable of splitting the &-1, 4-glycosidic bonds in pectic substances by a transeliminative mechanism in which the hydrogen from carbon 5 within the methylgalacturonate moeity was removed, thus leaving galacturonide residues having an unsaturated bond between carbons 4 and 5 of the reaction products. Such unsaturated reaction products usually absorb light strongly at 235 nm (Albersheim and Killias, 1962). Ability of some fungal enzymes to split pectic substances by transeliminative and hydrolytic mechanisms has been reported (Sherwood, 1966; Ayers and Papavizas, 1965; Spalding, 1969).

Penicillium sclerotigenum is a phytopathogen associated with the soft rot of yam (Discorea spp.) tubers in storage (Ogundana, Naqvi and Ekundayo, 1970). Plant cells are rich in pectic substances (Joslyn, 1962; Talmadge, Keegstra, Bauer and Albersheim, 1973. In addition yam tubers contain large amounts of starch. In a previous work, ability of *P. sclerotigenum* to secrete starch-degrading enzymes was reported (Olutiola, 1980). The present work examines the production of polygalacturonase and pectin lyase in culture filtrates of *P. sclerotigenum*. Some characteristics of the enzymes are also reported.

MATERIALS AND METHODS

Organism and culture conditions: The isolate (UNIFE. 03) of *Penicillium sclerotigenum* Yamamoto used was from the culture collection of the Mycological Habarium, University of Ife, Nigeria. It was isolated from rotten yam tubers by Dr. S. K. Ogundana of this Department. The organism was routinely grown and maintained on one per cent (w/v) malt yeast extract agar. The basal medium and the inoculation techniques were as previously described (Olutiola, 1976). Each 250 ml Erlenmeyer flask containing 100 ml of the synthetic medium was inoculated with 1 ml of an aqueous spore suspension containing approximately 5 x 10^5 spores per ml. Inoculated media were incubated without shaking at 30°C.

Preparation of culture filtrates: Preliminary experiments showed that production of polygalacturonase and pectin lyase by *P. sclerotigenum* was maximum after incubation for 168 hours. Filtrates from 168-h old cultures were clarified by centrifugation at 15,000 g for 10 min at 4°C. The filtrate was concentrated by ultrafiltration using UM-10 Diaflo membranes contained in LP-1A Amicon filtration apparatus. Protein content was determined by the method of Lowry, Rosebrough, Farr and Randall (1951). Mycelial mats and *Pencillium* infected yam tissues were extracted for polygalacturonase and pectin lyase activities as already described (Olutiola and Akintunde, 1979) except that the extraction solution was 0.1 M Tris-HCI buffer (pH 7.5 containing 0.5M NaCI, since this solution was more effective than a solution of 0.5M NaCI alone.

Enzyme separation: A column (25 x 640 mm) of Sephadex G – 100, surrounded by a water jacket maintained at 4°C, was prepared and calibrated as previous described (Olutiola, 1976). Ten ml of the enzyme concentrate were applied to the column and eluted with 0.1 M Tris-HC1 buffer (pH 7.5) containing 0.1 M KC1). The protein content of the eluted fractions was recorded by continous measurement at 280 nm, and fractions (5 ml per tube) were collected in an automated fraction collector (LKB, 7000 A Ultra Rac).

Fractions (26 to 32) which showed appreciable polygalacturonase and pectin lyase activitis were subjected to ammonium sulphate precipitation (80% saturation) and dialysed for 24 h against six changes of 0.1 M Tris-HC1 buffer (pH 7.5) at 4°C. Ten ml of the dialysate was applied to a column (25 x 340 mm) of DEAE-Sephadex A – 50 surrounded by a water jacket at 4°C and equilibrated with the same Tris-HC1 buffer.

Fractions(5 ml per tube) were collected and measured as described above. Each fraction was analysed for polygalacturonase and pectin lyase activity.

Assay Methods:

Polygalacturonase (PG) activity: Polygalacturonase activity was determined by measuring the reduction in viscosity of one per cent pectin (Sigma) and occasionally by determining increase in reducing groups liberated at 30°C. Viscometric measurements were made at 30°C in standard U-tube viscometers (BS/U, size C) containing 9 ml of one per cent pectin in 0.1 M sodium phosphate buffer (pH 5.0) and 1 ml enzyme preparation. Polygalacturonase activity was expressed as relative activity (RA). Relative activity was defined as 10^3 multiplied by the reciprocal of the time in minutes necessary for 50 per cent reduction in viscosity of the substrate at pH 5.0 at 30°C. Specific activity was defined as the relative activity units per mg protein. When polygalacturonase activity was measured by estimating the reducing sugars released, the reaction mixture contained 0.5 ml enzyme preparation and 4.5 ml of 1 per cent pectin in 0.1 M sodium phosphate buffer (pH 5.0). The reducing sugars released were measured by the method of Miller (1959). One unit of activity was defined as the amount of enzyme which liberated 1 mole of galacturonic acid in one minute under the assay conditions.

Pectin lyase (PL) activity:

Reaction products of the trans-eliminase reaction were detected by a modification of the thiobarbituric acid procedure of Weissbach and Hurwitz (1959) and by a direct spectrophotometric measurement of increasing absorbance at 235 nm caused by unsaturated bonds formed during the degradation of pectic substances (Sherwood, 1966). The reaction mixture and the method of analysis for the thiobarbituric acid assay and the direct spectrophotometric technique were as previously described (Olutiola and Akintunde, 1979). One unit of pectin lyase activity was defined as that amount of enzyme which caused an increase of 0.01 in absorbance at 540 nm or 235 nm per hour under the specified assay conditions of the tests. When the presence of polygalacturonase and pectin lyase activities were to be examined simultaneously the thiobarbituric acid reagent was used (Ayers *et al.* 1966). A peak at 510 – 515 nm was taken as being suggestive of the presence of polygalacturonase activity, while a peak at 547-550 was indicative of pectin lyase activity.

Tissue maceration and cellular death: Ability of *P. sclerotigenum* to macerate plant tissues was examined by the technique of Olutiola and Akintunde (1979) using discs of potato and yam tissues.

Cellular death was determined by setting up experiments as employed for tissue maceration (Olutiola and Akintunde, 1979). After incubation, toxicity was estimated by the technique of Tribe (1955). The test solution consisted of 10 ml 1 M KNO_3, 1 ml 0.1% neutral red chloride and 1 ml of M Tris-HCl buffer (pH 8.5). The discs were allowed to remain in the test solution for 2 min before being washed with 1 M KNO_3. Cells which still retained the neutral red after the wash were termed live cells while those which lost the stain were considered dead.

RESULTS

Growth and release of polygalacturonase and pectin lyase:

Penicillium sclerotigenum grew in synthetic liquid medium containing pectin as the sole carbon source. Growth of the organism increased with incubation period until the 168th hour, after which no further increase in growth was observed (Fig. 1). During growth, proteins which were capable of degrading pectic substances were released. Protein content in the medium paralleled mycelial dry weight, with both processes achieving maximum on the 168 h, beyond which no further increase was observed. During this period the pH of the medium became increasingly alkaline from an initial pH of 5.5 to a maximum pH of 7.0 on the 6th day.

When the culture filtrate was incubated with pectin, the ultraviolet absorption spectra of reaction products showed an increasing absorption at 235 nm. The ultraviolet absorption maximum near 230 – 235 nm is characteristic of unsaturated compounds released from pectic substances by transeliminase activity (Albersheim and Killias 1962). Spectrophotometric analysis of the reaction products of pectin, incubated with culture filtrate of *P. sclerotigenum*, exhibited absorption maximum at 550 nm at pH 8.5. Thus, spectra of thiobarbituric acid reaction products confirmed the formation of unsaturated galacturonyl products resulting from the transeliminative cleavage of pectin by culture filtrates of *P. sclerotigenum*.

The culture filtrate caused rapid reduction in viscosity of buffered pectin at pH 5.0. There was an increase in reducing power of the reaction mixtures which corresponded to loss in viscosity of the substrate. At pH 5.0, spectrophotometric determination of the reaction products of pectin with thiobarbituric acid reagent exhibited an absorption maximum at 510 nm. All these characteristics are indicative of presence of polygalacturonase activity in culture filtrates of *P. sclerotigenum* (Ayers *et al.* 1966; Talboys and Busch, 1970).

Growth and production of pectic enzymes on different carbon sources: Experiments were carried out to examine the constritutive or inductive nature of the polygalacturonase and pectin

lyase produced by *P. sclerotigenum*. The organism was grown in media containing sucrose, glucose, maltose pectin, sodium polypectate, polygalacturonic acid, yam slices and some other carbon sources (Table 1). Culture filtrates of the organism grown on carboxymethylcellulose, mannitol, glucose, sucrose, lactose, maltose or inositol lacked Polygalacturonase and pectin lyase activities (Table 1). However the organism grew quite well and released proteins into the medium on each of these carbohydrates. When the organism was grown in media containing pectin, sodium polypectate, polygalacturonic acid or yam slices, appreciable quantities of proteins which exhibited polygalacturonase and pectin lyase activities were released into the medium.

Fractionation on Sephadex G – 100: Fractionation of the 2nd ammonium sulphate precipitate (Table II) on Sephadex G – 100 produced a single absorption peak designated A (Fig. 2). The molecular weight of this component was approximately 72,000. Further separation of this component on DEAE – Sephadex A – 50 column resulted in three peaks of absorption designated Aa, Ab, and Ac. Components Aa and Ab exhibited polygalacturonase activity while components Aa, Ab and Ac possessed pectin transeliminase activity (Fig. 3).

Properties of the partially purified polygalacturonase and pectin lyase: The partially purified components of peak A (Fig. 3) were employed.

Maceration and cellular death: The partially purified polygalacturonase and pectin lyase of *P. sclerotigenum* caused maceration and cellular death of potato and yam tissues. Similar maceration and cellular death of host cells have been attributed to the degradation of the host tissue by pectic enzymes (Garibaldi and Bateman, 1971; Tribe, 1955).

Effect of some chemicals: The enzyme preparation was incubated with different concentrations of $CaCl_2$, NaCl, Ethylenediamine tetra-acetic acid (EDTA) and 2, 4 – dinitrophenol (DNP) respectively for 1 hour at 4^oC, and aliquots were removed and used in enzyme assays. Activities of polygalacturonase and pectin lyase of *P. sclerotigenum* were stimulated by Ca^{++} and Na^+, but inhibited by EBTA and DNP (Table III).

Effect of pH: Optimum activity of polygalcturonase occurred at pH 5.0, and that of pectin transeliminase was at pH 8.5 (Fig. 4). Thus acidic conditions were more favourable to polygalacturonase of *P. sclerotigenum* while alkaline conditions favoured pectin lyase activity Pectin lyase activity was not obtained below pH 5.0. Extract of yam infected by *P. sclerotigenum* has a pH of approximately 7.0, while the pH of extract from uninfected yam is approximately 6.5. Activity of polygalacturonase and pectin lyase occurred at both pH levels (Fig. 5). Maximum activity of polygalacturonase and pectin lyase occurred at 35^oC (Fig. 5). There was a rapid loss of activity of both enzymes at 50^oC.

Effect of pectin concentration: Polygalacturonase and pectin lyase activities increased with increase in concentration of pectin in the reaction mixtures, achieving saturation at pectin concentrations of 9 mg/ml and 11 mg/ml for pectin lyase and polygalacturonase respectively.

DISCUSSION

Penicillium sclerotigenum secreted extracellular enzymes which degraded pectic substances hydrolytically and in a transeliminative fashion. Polygalacturonase and pectin lyase enzymes were detected in the culture filtrates of *P. sclerotigenum* grown in media containing pectic substances. Activities of both enzymes were stimulated by cations and inhibited by ethylendiamine tetra-acetic acid (a metal-chelating agent), suggesting that metal ions are likely to play essential role in the activity of these enzymes. Cation stimulation and EDTA inhibition of fungal pectinases have been reported by some workers (Sherwood, 1966; Wang and Pinckard, 1971; Hagar and McIntyre, 1972). Inhibition of the activities of both enzymes by 2, 4-dinitrophenol, an uncoupling agent which interferes with aerobic phosphorylation processes, suggests the

importance of an energy-yielding process in the activities of the enzymes (Dixon and Wabb, 1971) Besides their presence in culture filtrates, polygalacturonase and pectin lyase activities were detected in extracts of fungal mycelium as well as in extracts of Penicillium – infected yam tissues. Thus both enzymes were produced by *P. sclerotigenum in vitro* and *in vivo*. The enzymes were absent in healthy yam tissues, indicating that both enzymes were of fungal origin.

Production of polygalacturonase and pectin lyase by *P. sclerotigenum* occurred only when a pectic substance was present in the medium (Table 1), suggesting the inductive nature of both enzymes. It is believed that the inductive synthesis of enzymes by a microorganism confers on the parent organism, an important biological control mechanism whereby the cells' equipment of enzymes is adjusted to the needs of its metabolism (Rose, 1968; Wiseman and Gould, 1971). Synthesis of polygalacturonase and pectin lyase by *P. sclerotigenum* could be of advantage, as the organism will be equipped with enzymes necessary for both hydrolytic and transeliminative cleavage of the &, 1-4-glycosidic bonds of pectic substances contained in the host tissue. Many workers have implicated polygalacturonase and pectin lyase in pathogenesis (Mount *et al.* 1970; Fanelli, Cacace and Cervone, 1978; Lisker, Katan and Henis, 1975).

LITERATURE CITED

ALBERSHELM, P. and KILLIAS, U. 1962. Studies relating to the purification and properties of pectin transeliminase. *Arch. Biochem. Biophys. 97:* 107 – 115

AYERS, W. A. and PAPAVIZAS, G. C. 1965. An exocellular pectolytic enzyme of *Aphanomyces euteiches. Phytopathology* 55: 249 – 253.

AYERS, W. A. PAPAVIZAS, G. C. and DIEM, A. F. 1966. Polygalacturonate transeliminase and Polygalacturonase production by *Rhizoctonia solani. Phytopathology 56:* 1006 – 1011.

DIXON, M. & WEBB, E. C. 1971. *Enzymes.* William Clowes and Sons Ltd., Great Britain.

FANELLI, C. CACACE, M. G. and CERVONE, F . 1978. Purification and properties of two polygalacturonases from *Trichoderma koningii.* J. Gen. Microbiol. 104: 305 – 309.

GARIBALDI, A. and BATEMAN, D. F. 1971: Pectic enzymes produced by *Erwinia Chrysanthemi* and their effects on plant tissue. *Physiol. Pl. Path. 1 :* 25 – 40.

HAGAR, S. S. and McINTYRE, G. A. 1972. Pectic enzymes produced by *Pseudomonas fluorescens,* an organism associated with "pink eye' disease of potato tubers. *Can. J. Bot. 50:* 2479 – 2488.

HSU, E. J. and VAUGHN, R. H. 1969. Production and catabolite repression of the constitutive polygalacturonic acid transeliminase of *Aeromonas liquefaciens. J. Bacteiol. 98:* 172 – 181.

JOSLYN, M. A. 1962. The chemistry of protopectin: a critical review of historical data and recent developments. *Adv. Food Res. 11:* 1 – 105.

LISKER, N. Katan, J. and HENIS, Y. 1975. Sequential production of polygalacturonase, cellulase, and pectin lyase by *Rhizoctonia solani. Can. J. Microbiol. 21:* 1298 – 1304.

LOWRY, O. H. ROSEBROUGH, N. J. FARR, A. L. and RANDALL, R. J. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem. 193:* 265 – 275.

LUND, B. M. and BROCKLEHURST, T. F. 1978. Pectic enzymes of pigmented strains of Clostridium. *J. Gen. Microbiol. 104:* 59 – 66.

MILLER, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analyt. Chem. 31:* 426 – 428.

MOUNT, M. S. BATEMAN, D. F. and BASHAM, H. G. 1970. Induction of electrolyte loss, tissue maceration, and cellular death of potato tissue by an endopolygalacturonate trans-eliminase. *Phytopathology 60:* 924 – 931.

OGUNDANA, S. K. NAQVI, S. H. Z. and EKUNDAYO J. A. 1970. Studies on fungi associated with soft rot of yams (Discorea spp.) in storage in Nigeria. *Trans. Brit. Mycol. Soc. 54:* 435 – 444.

OLUTIOLA, P. O. 1976. A cellulase complex in culture filtrates of *Penicillium citrinum. Can. J. Microbiol 22:* 1153 – 1159.

OLUTIOLA, P. O. • 1980. Extracellular &-amylase production by the yam (Discorea spp.) rot organism, *Penicillium solerotigenum Yamamoto. Acta Phytopathol. Acad. Sci Hung.* (In Press).

OLUTIOLA, P. O. and AKINTUNDE, O. A. 1979. Pectin lyase and pectin methylęsterase production by *Penicillium citrinum. Trans. Br. Mycol. Soc. 72:* 49 – 55.

ROSE, A. H. 1968. *Chemical Microbiology.* Butterworths, Great Britain.

SHERWOOD, R. T. 1966. Pectin lyase and Polygalacturonase production by *Rhizoctonia solani* and other fungi. *Phytopathology* 56 : 279 – 286.

SPALDING, D. H. (1969). Toxic effect of macerating action of extracts of sweet potatoes rotted by *Rhizopus stolonifer* and its inhibition by ions. *Phytopathology* 59, 685 – 692.

TALBOYS, P. W. and BUSCH, L. V. 1970. Pectic enzymes produced by *Verticillium* species. *Trans. Br. Mycol. Soc. 55:* 367 – 381.

TALMADGE, K. W. KEEGSTRA, K. BAUER, D. D. and ALBERSHEIM, P. 1973. The structure of plant cell walls. 1. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. *Plant Physiol. 51:* 158 – 173.

TRIBE, H. T. 1955. Studies on the physiology of parasitism XIX. On the killing of plant cells by enzymes from *Botrytis cenerea* and *Bacterium aroideae. An. Bot.* (N.S.) *19:* 351 – 371.

WANG, S. C. and PINCKARD, J. A. 1971. Pectin enzymes produced by *Diplodia gossypina in vitro* and in infected cotton bolls. Phytopathology 61: 1118 – 1124.

WEISSBACH, A. and HURWITZ, J. 1959. The formation of 2-keto-3-deoxy-heptonic acid in extracts of *Escherichia Coli. J. Biol. Chem. 234:* 705 – 709.

WISEMAN, A. & GOULD, B. J. 1971. *Enzymes, their nature and role.* Hutchinson Educational Ltd. London.

WOOD, R. K. S. 1967. *Physiological Plant Pathology.* Blackwell, Oxford.

TABLE 1

Effect of carbon source on production of polygalacturonase (PG) and pectin lyase (PL) activities by *P. sclerotigenum*.

Carbon source 1% (w/v)	Mycelial dry wt (mg)	Protein in medium (ug/ml)	PG (Units/ml)	Pl (Units/ml)
	Mean S.E.*	Mean S.E.	Mean S.E	Mean S.E
Pectin	132.8±1.5al**	215.7±2.3a	45.3±0.9b	19.2±0.5a
Yam	348.2±3.5b	480.6±3.1b	39.7±1.5c	5.5±0.1d
Carboxymethylcellulose	22.7±1.0c	75.0±0.8c	0.0e	0.0e
Mannitol	92.2±2.5dm	110.5±1.0d	0.0e	0.0e
Glucose	164.5±3.0e	270.3±1.1e	0.0e	0.0e
Sucrose	174.7±1.5f	310.9±0.7f	0.0e	0.0e
Lactose	39.6±0.9gn	93.5±1.5g	0.0e	0.0e
Maltose	97.4±1.1hm	150.1±0.5h	0.0e	0.0e
Inositol	35.5±2.0in	81.4±0.7il	0.0e	0.0e
Sodium polypectate	139.5±0.8jl	220.6±0.5j	46.9±0.2a	16.3±0.90b
Polygalacturonic acid	33.7±0.5kn	79.5±1.1kl	10.5±0.1d	6.3±0.2c

*SE = Standard error

** Means followed by the same letter in each column are not significantly different (5% level, Duncan's multiple range test).

TABLE II

Partial purification of polygalacturonase and pectin lyase of *P. sclerotigenum*

	Polygalacturonase				
Fraction	Total Activity (units)	Total protein (mg)	Specific activity units/mg protein	Yield (%)	Purification (fold)
Crude culture filtrate	117500	525	223.9	100	–
Concentration (UM-10)	47000	135	348.1	40	1.6
1st $(NH)_2SO_4$ precipitation (60 –·90%)	36154	47	769.2	31	3.4
Sephadex G–100	30128	15.2	1982.1	26	8.9
2nd $(NH_4)_2SO_4$ precipitation (90 %)	18830	6.6	2853.0	16	12.7
DEAE-Sephadex A–50					
Peak					
Aa	6091	1.5	4060.7	5	18.1
Ab	3205	1.1	2913.6	3	13.0
Ac	–	–	–	–	–

		Pectin lyase		
Total activity (units)	Total protein (mg)	Specific activity units/mg protein	Yield %	Purification (fold)
46250	525	88.1	100	–
22024	135	163.1	48	1.9
20022	47	426.0	43	4.8
18202	15.2	1197.5	39	13.6
14001	6.6	2121.4	30	24.1
3515	1.5	2343.3	8	26.6
3400	1.1	3090.9	7	35.1
4015	1.2	3345.8	9	38.0

TABLE III

Influence of some chemicals on the activities of partially purified (gel filtration, Sephadex G-100) polygalacturonase (PG) and pectin lyase (PL) obtained from 168-h-old culture filtrates of p. *P. sclerotigenum*

Concentration of chemical (mM)	Enzyme activity as percentage of control							
	$CaCl_2$		NaCl		EDTA		DNP	
	PG	PL	PG	PL	PG	PL	PG	PL
0*	100e*	100e	100e	100c	100a	100a	100a	100a
1	105d	119d	102d	100c	33b	45b	41b	49b
5	109c	125c	107c	101c	10c	23c	25c	30c
10	117b	133b	121b	105b	2d	11d	9d	14d
20	122a	140a	125a	110a	0e	0e	0e	0e

*No Chemical added, i.e. control

**Means followed by a different letter in each column are significantly different (5% level, Duncan's multiple range test).

LIST OF FIGURES

Fig. 1. Growth of *P. sclerotigenum* (as total mycelial dry weight,(□) in liquid medium containing 1 % pectin (w/v), showing pH (■) and protein content (●) in the medium, and activities of polygalacturonase (0–0–0, percentage reduction determination; 0–0–0–, reducing sugars analysis) and pectin lyase △-△-△ , thiobarbituric acid reaction at 540 nm; △–△–△ direct spectrophotometry at 235nm).

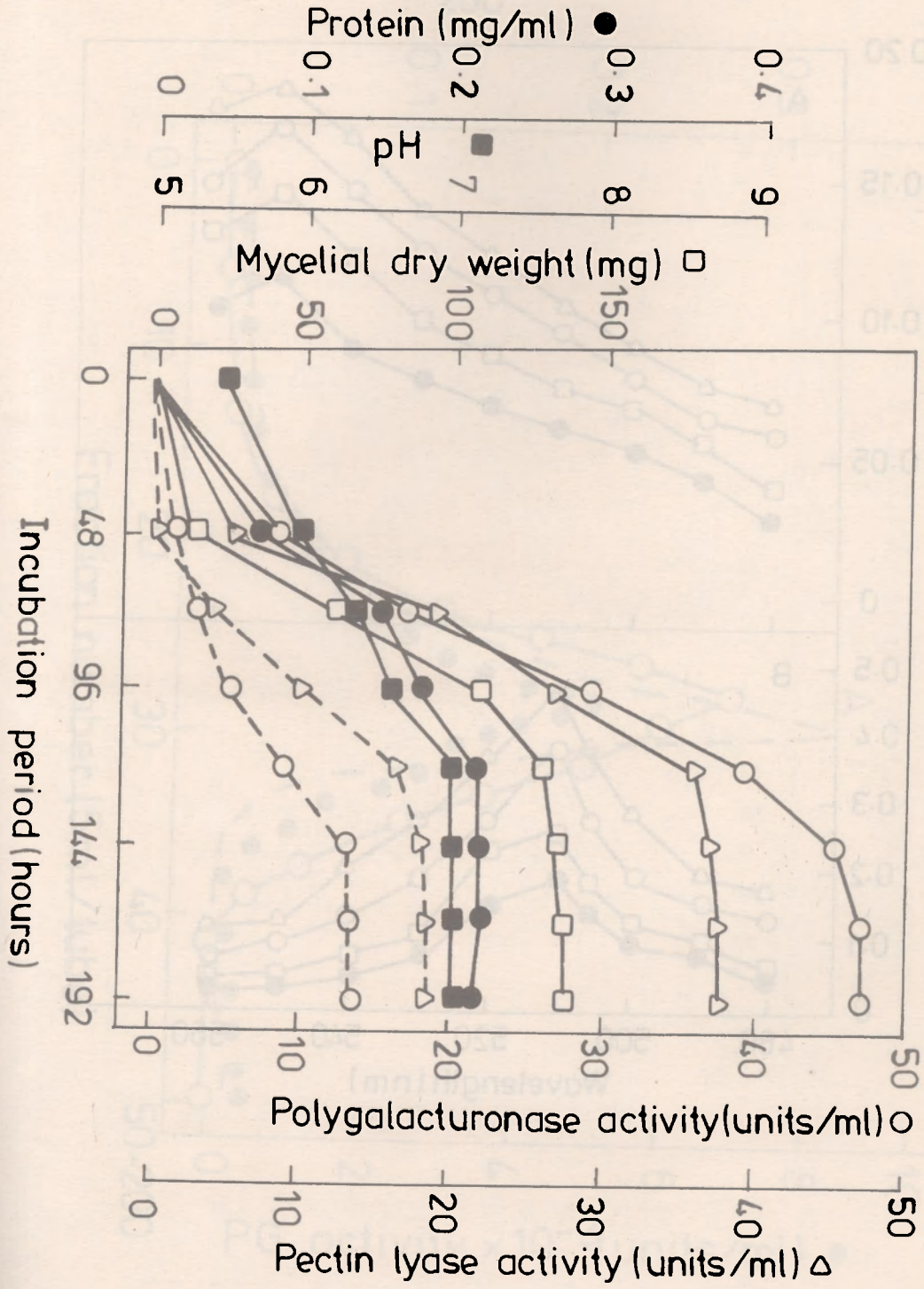
Fig. 2. Separation of proteins in concentrated culture filtrates of *P. sclerotigenum* by gel filtration, and polygalacturonase (PG) and pectin lyase (PL) activities of the fractions. 0–0–0, protein (E_{280}); ●–●–●, polygalacturonase activity; – – –, pectin lyase activity.

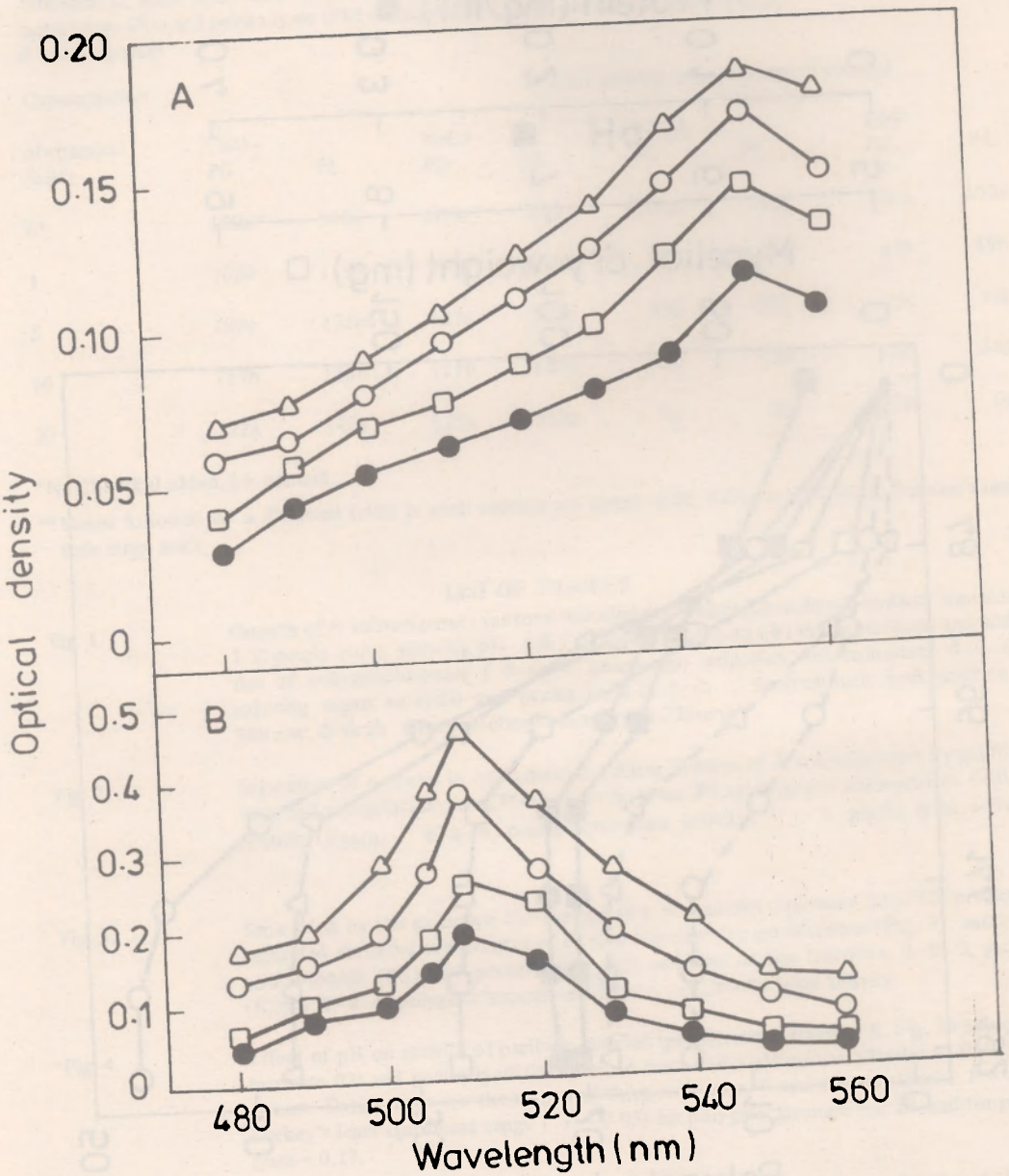
Fig. 3. Separation by ion exchange chromatography of proteins (fractions 26 – 32) previously separated from culture filtrates of *P. sclerotigenum* by gel filtration (Fig. 3), and polygalacturonase (PG) and pectin lyase (PL) activities of the fractions. 0–0–0, protein (E280); ●–●–●, polygalacturonase activity; – – –, pectin lyase activity.

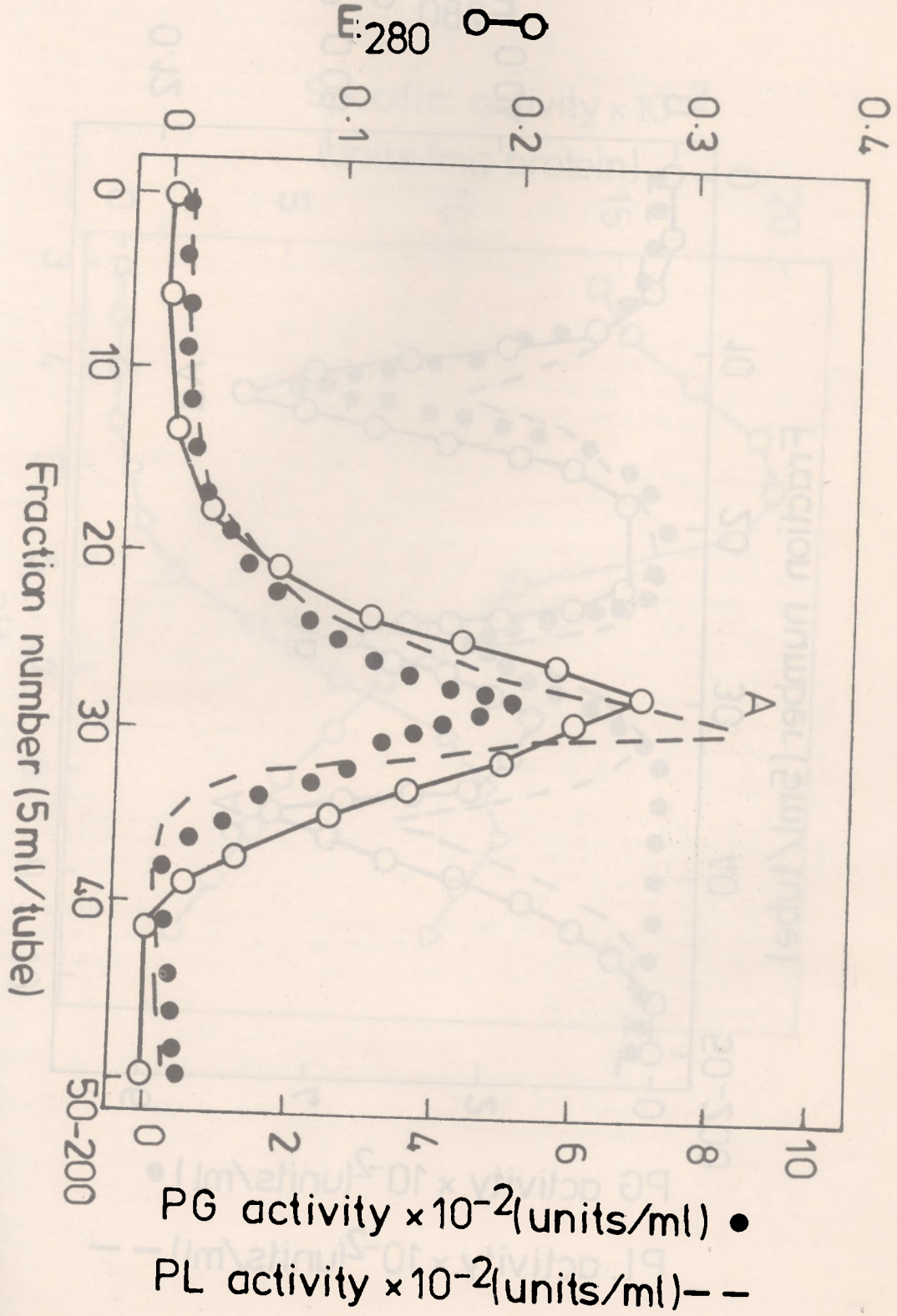
Fig. 4. Effect of pH on activity of partially purified (gel filtration, fraction A, Fig. 3) polygalacturonase (O) and pectin lyase (Δ) obtained from 168-h-old culture filtrates of *P. sclerotigenum*. Data points are the means of three replicates. F test was significant, P = 0.01 Turkey's least significant range (P = 0.05) for polygalacturonase = 0.29, and for pectin lyase = 0.17.

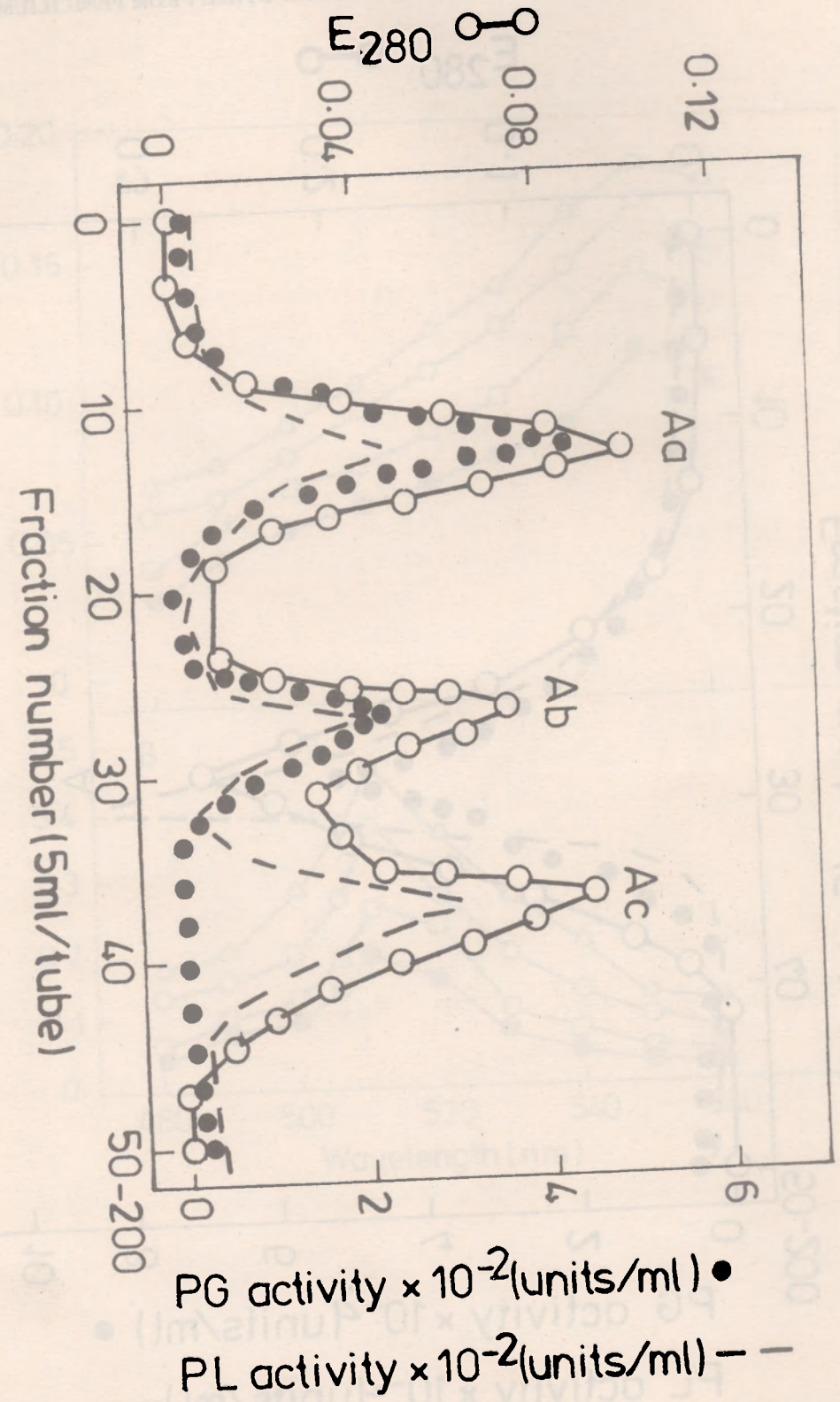
Fig. 5. Effect of temperature on activity of partially purified (gel filtration, fraction A, Fig.3) polygalacturonase (O) and pectin lyase (Δ) obtained from 168-h-old culture filtrates of *P. sclerotigenum*. Data points are the means of three replicates. F test was significant, P = 0.01. Turkey's least significant range (P=0.05) for polygalacturonase - 0.20 and for pectin lyase = 0.49.

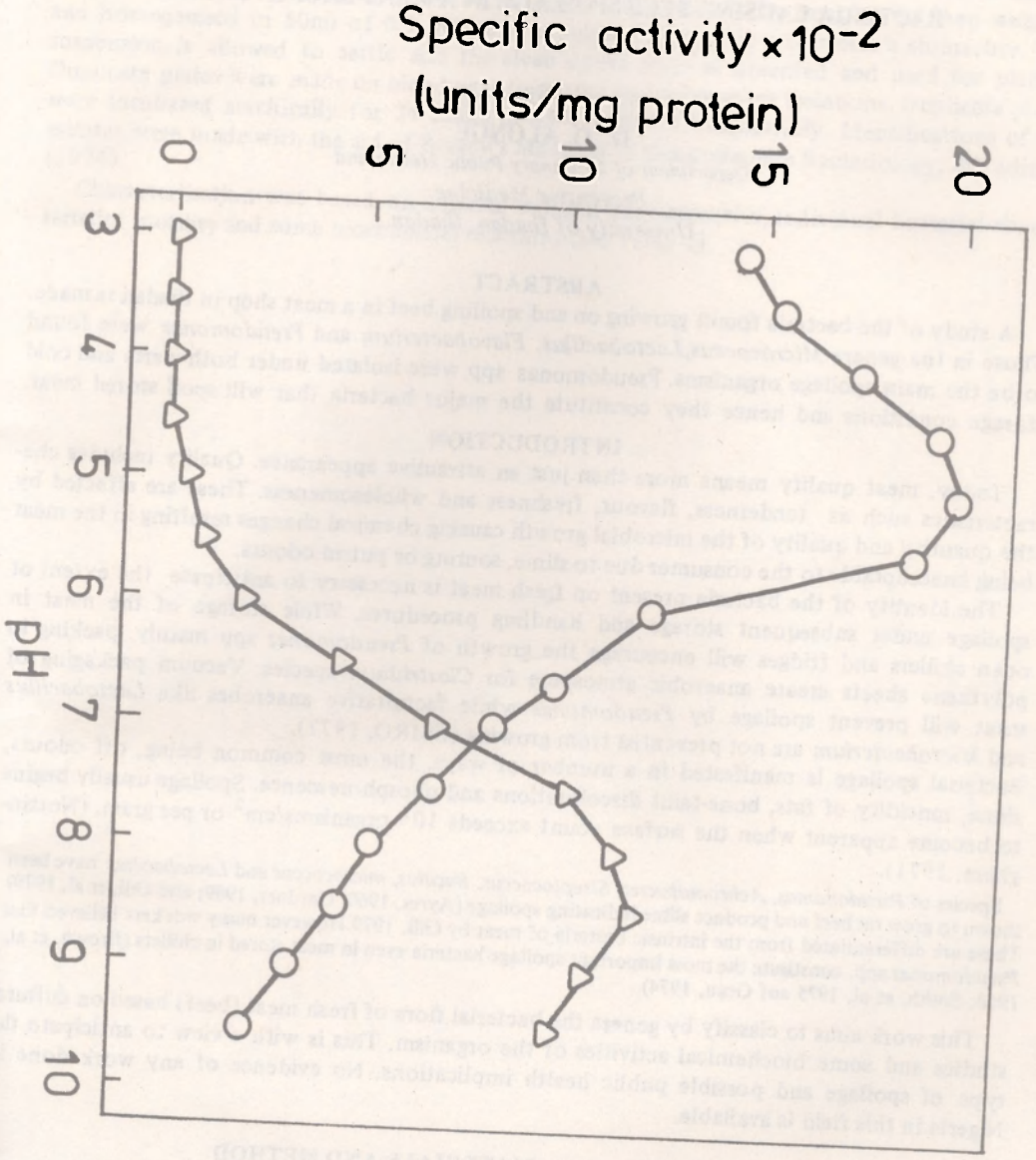
Incubation period (hours)


Protein (mg/ml) ●
0
0·1
0·2
0·3
0·4
pH ■
5
6
7
8
9
Mycelial dry weight (mg) □
0
50
100
150
Incubation period (hours)
0
48
96
144
192
0
10
20
30
40
50
Polygalacturonase activity (units/ml) ○
0
10
20
30
40
50
Pectin lyase activity (units/ml) △


0·20
0·15
0·10
0·05
0
A
Optical density
0·5
0·4
0·3
0·2
0·1
0
B
480
500
520
540
560
Wavelength (nm)


E280
0
0.1
0.2
0.3
0.4
0
10
20
30
40
50-200
Fraction number (5ml/tube)
A
0
2
4
6
8
10
PG activity x 10⁻²(units/ml) •
PL activity x 10⁻²(units/ml)– –


E280
0
0·04
0·08
0·12
0
10
20
30
40
50-200
Fraction number (5ml/tube)
Aa
Ab
Ac
0
2
4
6
PG activity x 10-2(units/ml) •
PL activity x 10-2(units/ml) – –


Specific activity x 10-2
(units/mg protein)
0
5
10
15
20
pH
3
4
5
6
7
8
9
10