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MICROBIAL DECOMPOSITION OF PECTIC SUBSTANCES – IV

Bacteria Decomposing Pectin with particular reference to some *Xanthomonas sp.*

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ABSTRACT

Out of the 313 bacterial cultures surveyed for pectinolytic activity 70 were found pectinolytic. The enzymes make-up of these pectinolytic bacteria consisted generally of polygalacturonase, pectin methylestrase and *trans*-eliminase systems. All the bacterial pectinases were found to be adaptive in nature.

A hitherto unknown mode of attack on the polygalacturonate by *Xanthomonas* pectinase is suspected in as much as a reducing substance (*s*) other than D-galacturonic acid is formed in this system. Some studies regarding the growth of the *Xanthomonas sp.* and elaboration and inhibition of pectinase produced by it were also carried out.

INTRODUCTION

With the report of Alberstein *et al*[1] on the degradation of pectin by a *trans*-elimination mechanism, there has been a spurt of research activity in the field of microbial pectinolytic enzymes. While there is no doubt about the existence in moulds and yeasts of pectinolytic systems containing a true polygalacturonase, many bacteria which were earlier believed to elaborate polygalacturonase do not seem to possess it notwithstanding the fact that they can attack pectic substances. According to Hasegawa and Nagel[2], all polygalacturonases may well be *trans*-eliminases. The purpose of this paper

is to show that while most bacterial systems comprise of *trans*-eliminases there exists a possibility of other modes of attack, *e.g.*, the one witnessed in four members of the genus *Xanthomonas* worked out in this laboratory.

MATERIALS AND METHODS

Bacterial cultures. The large number of bacteria surveyed for pectinolytic activity included freshly isolated cultures from pectin enrichments, as well as bacteria maintained in the culture collection of this laboratory. The cultures were maintained on agar slants of two media, one containing 0.5 % pectin and the other without pectin (Haynes *et al*[3]).

Identification. Methods followed for the identification of the bacteria were those described in the Manual of Microbiological Methods[4]. Bergey's manual[5] and the paper by Borman *et al*[6] were consulted for identifying the bacteria upto generic level.

Pectinolytic activity. The medium employed for the survey as well as the production of bacterial pectinases consisted of 0.5 % pectin, 0.1 % yeast extract (Difco) and basal salts solution. The methods of Bell and Etchell[7] were followed for the detection in general of pectinolytic activity. The polygalacturonase activity of representative cultures was ascertained by the method of Jansen and MacDonnell[8]. The pectin methylesterase activity was followed by titrating the carboxyl groups produced[9], while that of *trans*-eliminase by the method of Nagel and Vaughn[10]. Pectin was estimated by the calcium pectate method of Kertesz[11].

Preparation of crude pectinases. The exocellular enzymes of bacteria were obtained in the form of crude powder by precipitation with cold acetone or saturated ammonium sulphate. The precipitate in either case was centrifuged off and dialyzed against distilled water. An acetone powder of bacterial cells was prepared using the method of Umbreit *et al*[12] for further investigations.

Chromatographic examination of reaction mixtures. Paper chromatography, both circlular and descending, was employed to elucidate the course of the enzyme reaction. Whatman No. 1 paper, ethyl acetaet-pyridine-water-acetic acid (5 : 5 : 3 : 1) solvent system, and either 0.5 % benzidine reagent or aniline-trichloroacetic acid reagent were used for detecting uronic acid spots. The chromatograms were run for 12 hr. 10 μ l aliquots of a 1 % D-galacturonic acid were always spotted for comparative purposes. Tests for suspected D-galacturonic acid spots were carried out by using cysteine napthoresorcinol and lead acetate reagents.

Study on Xanthomonas pectinase. All the four strains of *Xanthomonas sp.* (No. 40, 43, 107 and 110 from the culture collection) were first tested for their pectinase activity by the Jansen and MacDonell method[8]. Then the culture filtrates of *Xanthomonas sp.* (No. 110) obtained from shake cultures after 1, 2, 4 and 7 days and from stationary cultures after 2, 4, 7 and 10 days were

examined in detail. The effect of incorporating $CaCO_3$ in the medium was also studied both in the shake and stationary cultures on the 7th day. The endo- and exo-pectinases from this strain were prepared and their effect on pectin and polygalacturonate as substrates in the presence and absence of calcium ions was followed.

Results and Discussion

The results of the survey of pectinolytic activity among the bacteria tested are summarised in Table I. From a glance it will be seen that whereas genera

TABLE I

Pectinolytic activity* among certain bacterial sp.

Bacterial cultures	Number tested	Number pectolytic	Bacterial cultures	Number tested	Number pectolytic
Achromobacter sp.	6	Nil	*Escherichia freundii*	3	3
Aerobacter aerogenes	8	1	*Flavobacterium* sp.	13	10
Aerobacter cloacae	4	Nil	*Micrococcus* sp.	1	Nil
Alcaligenes sp.	1	Nil	*Mycobacterium lacticola*	2	Nil
Alcaligenes faecalis	1	Nil	*Paracolobactrum aerogenoides*	6	2
Arthrobacter sp	19	1			
Azotobacter agiles	9	Nil	*Paracolobactrum coliform*	6	6
Bacillus sp.	3	Nil	*Paracolobactrum intermedium*	1	1
Bacillus brevis	1	Nil			
Bacillus cereus	3	Nil	*Proteus inconstans*	1	Nil
Bacillus megaterium	4	Nil	*Proteus morganii*	3	Nil
Bacillus subtilis	8	8	*Pseudomonas* sp.	29	Nil
Bacillus sphaericus	2	Nil	*Pseudomonas aeruginosa*	14	Nil
Brevibacterium sp.	14	2	*Pseudomonas convexa*	4	Nil
Brevibacterium ammoniagenes	3	Nil	*Pseudomonas fermentans*	2	Nil
Colobactrum aerogenes	8	1	*Pseudomonas helianthi*	3	Nil
Colobactrum coli	1	1	*Pseudomonas maublancii*	1	Nil
Colobactrum freundii	1	Nil	*Pseudomonas stutzeri*	2	Nil
Corynebacterium sp.	1	Nil	*Pseudomonas vitiswoodrowii*	2	Nil
Corynebacterium barkeri	7	7	*Sarcina* sp.	1	Nil
Corynebacterium carotenogenum	3	Nil	*Serratia marcescens*	3	Nil
			Xanthomonas sp.	22	16
Corynebacterium laevaniformans	9	Nil	*Xanthomonas axonopodis*	4	Nil
			Xanthomonas panici	6	Nil
Erwinia sp.	5	4	Unidentified	25	6
Erwinia dissolvens	1	1			
Escherichia coli	1	Nil	Total	313	70

* Pectinolysis was cheked by the method of Bell & Etchell[7].

such as *Bacillus. Corynebacterium, Erwinia, Flavobacterium* and *Xanthomonas* were pectinolytic, no pectinolysis was detected in others, *e.g., Pseudomonas.* This observation was somewhat conflicting when seen in juxtaposition with previous observation of Betrabet[13]and Betrabet and Bhat[14] who found members of this genus dominating in the rets of *Malachra capitata* L. and *Hibiscus cannabinus* L. Reports from other laboratories[15-19] have also implicated the genus *Pseudomonas* in pectin decomposition. Since then the " pseudomonas ", which were shown to lose their power of pectinolysis on storage, were studied in detail and conditions favourable for retention of their pectinolytic abilities have been worked out (Agate and Bhat, unpublished data).

In order to detect pectinolysis even in weakly pectinolytic cultures quantitiative tests were found useful. The results of these experiments are summarised in Table II. From this table it will be clear that *Pseudomonas sp*

TABLE II

Bacterial decomposition of pactin as tested by the calcium pectate method *

Culture filtrate from	Period of growth (in days)	Calcium pectate values (in grams) and volume of culture filtrate tested
Experiment A		*in 2.5 ml.*
Ps. aeruginosa – PD	3	(i) 0.0206 *g*
C. aerogenes – G 7·0 (1)	3	(i) 0.0208 *g*
Uninoculated	3	(i) 0.0193 *g*
Experiment B		*in 10.0 ml.*
Ps. aeruginosa – EIE 6P3	9	(i) 0.0205 *g*
do	16	(i) 0.0207 *g*
Ps. convexa – EIE 6P5	9	(i) 0.0211 *g*
do	16	(i) 0.0207 *g*
Uninoculated	9	(i) 0.0218 *g*
do	16	(i) 0.0207 *g*
Experiment C		*in 2.5 ml.*
Erwinia sp – G· 7·0 (2)	1	(i) 0.0185 *g*
do	2	(i) 0.0063 *g*
do	3	(i) 0.0012 *g*
Erwinia sp – Cu 7·0 (4)	1	(i) 0.0303 *g*
do	2	(i) 0.0210 *g*
do	3	(i) 0.0126 *g*
Uninoculated	1	(i) 0.0334 *g*
do	2	(i) 0.0326 *g*
do	3	(i) 0.334 *g*
Experiment D		*in 10.0 ml.*
B. subtillis – 54	2	(i) 0.0020 *g*
Flavobacterium sp – 13D	2	(i) 0.0040 *g*
Uuinoculated	2	(i) 0.0207 *g*

* Pectin was estimated by the calcium pectate method of Kertesz[11].

which gave negative results in the earlier survery were definitely non-pectinolytic whereas the *Erwinia sp. B. subtilis* and *Flavobacterium sp* were able to decompose pectin. Two cultures of *Paracolobactrum coliform* were also found to decompose 28% (No. 23) and 58% (No. 24) of pectin in 48 hr. under stationary culture conditions.

Pectin methylesterase activity was exhibited by the genera *Corynebacterium* (6 strains of *C. barkeri*) and *Erwinia* [(Nos. G 7.0 (2) and G 7.0 (4)] whereas the 4 *Xanthomonas* strains were devoid of this activity. In the case of *Erwinia sp.* pectin in the medium appeared to be essential for the elaboration of the enzyme pectin methylesterase. Mills[20], who studied the pectin methylesterase of *Pseudomonas prunicola*, also described it as an exocellular adaptively formed enzyme.

Results of several bacterial cultures tested for their ability to split glycosidic bonds of the polygalacturonate molecule are summarised in Table III. From this table it will be seen that the pseudomonads encountered here were without action on the polygalacturonate molecule, while the *Erwinia sp.*, *C. barkeri* and *B. subtilis* could split glycosidic linkages of the molecule. It was seen during these experiments that pH 7.5 is more suited to pH 4.5 for the reactions.

TABLE III

Polygalacturonase activity of certain bacteria

Culture filtrate from 5—7 day	Increase in reducing power in terms of 0.05N $Na_2S_2O_3$ solution			
	6 hr.	18 hr.	26 hr.	48 hr.
Erwina sp – G. 7.0 (2)	1.35	.....	1.65	1.75
Erwina sp – Cu 7.0 (4)	0.65	.....	1.05	1.20
P. aeruginoasa – EIE 6P3	0.05	.....	0.10	0.10
P. convexa – EIE 6P5	Nil	.....	Nil	Nil
C. barkeri – 3 Pe	1.10	1.40	1.45	1.55
C. barkeri – 5 Pe	1.20	1.40	1.50	1.60
C. barkeri – 15 Pe	0.65	1.05	1.20	1.40
C. barkeri – 16 Pe	0.75	1.20	1.25	1.45
C. barkeri – 17 Pe	1.00	1.30	1.45	1.55
C. barkeri – 22 Pe	0.55	0.95	1.15	1.30
B. subtilis – " *Bread* "	0.15	0.45	.....	0.60
B. subtilis – 54	1.25	2.80	.....	3.50
Control	No change			

It was also evident from the experiments that the profuse elaboration by bacteria of pectinase occurred only when pectin was present in the growth medium. Medium without pectin permitted elaboration, at most, of only a trace of pectinase. The results obtained with *B. subtilis* (No. 54) and *Erwinia sp.* [G. 7.0 (2)] and presented in Table IV are typical cases in point. The bacterial cultures used herein were those maintained on glucose and for test inoculated into pectin yeast-extract mineral medium and glucose yeast-extract mineral medium. The culture filtrates from both were subsequently tested for the presence of pectinase at different stages of growth. It might be mentioned here that Kraght and Starr[21] as well as Ozawa and Okamoto[22] have reported previously on the inducible nature of the pectic enzmes of *Erwinia sp.*

In the case of *Xanthomonas sp.* examined, it was evident that increase in the reducing power of the reaction mixture continued even after all the polygalacturonic acid had been accounted for indicating thus the formation of other reducing substances and/or galacturonic acid. This is amply revealed from the results recorded in Table V and the subsequent calculatious made thereon. From the table it will be seen that the increase in reducing power corresponds to about 8.7 ml. of 0.05 N $Na_2S_2O_3$ solution, whereas the increase, if only galacturonic acid was produced, should have been 5.68 ml. of 0.05 N $Na_2S_2O_3$ solution. The effect of shaking and addition of $CaCO_3$ in the medium on *Xanthomonas* pectinase is presented in Table VI. Both these were observed to affect the exocellular pectinase production. The reasons for this are not known but it would be interesting to know if these would affect the endocellular pectinase production in a similar fashion.

TABLE IV

Polygalacturonase activity of *Erwinia* sp and *B. subtilis* grown in glucose and pectin media at 7.5 pH.

Bacterial culture filtrate tested	Growth medium containing	Increase in reducing power in terms of 0.05N $Na_2S_2O_3$ solution				
		1 hr.	4 hr.	12 hr.	24 hr.	45 hr.
Erwinia sp – G 7.0 (2) 6 days	Pectin	0.85	1.05	1.15	1.30	1.40
do do	Glucose	Nil	Nil	0.05	0.05	0.15
Erwinia sp – G. 7.0 (2) 11 days	Pectin	0.85	1.10	1.15	1.20	1.30
do do	Glucose	Nil	0.10	0.15	0.25	0.35
B. subtilis – 54 – 3 days	Pectin	0.30	0.65	0.90	1.10	----
do do	Glucose	Nil	Nil	0.05	0.10	.....
B. subtilis – 54 – 6 days	Pectin	0.25	0.80	2.20	2.95	.....
do do	Glucose	Nil	Nil	0.15	0.15	.....
Controls		No change				

TABLE V

Polygalacturonase activity of stationary cultures of *Xanthomonas* sp.

Culture filtrate from Isolate No.	pH of culture filtrate	Increase in reducing power in teams of 0.05N $Na_2S_2O_3$					
		10 hr.	10 hr.	36 hr.	60 hr.	70 hr.	93 hr.
40	5.9	0.30	1.40	5.85	8.25	8.70	8.65
43 – Sodium benzoate	5.8	0.30	1.90	7.35	9.15	9.25	8.80
43 – No sodium benzoate	5.8	0.30	2.00	7.75	9.20	9.25	8.70
107	5.9	0.25	1.40	6.00	8.30	8.75	8.80
110	5.8	0.10	2.30	8.30	9.40	9.20	8.75
Controls		Slight decrease in reducing power					

TABLE VI

Effect of shaking and incorporation of $CaCO_3$ on formation of polygalacturonase by *Xanthomonas* sp (No. 110)

Culture filtrates from	pH of culture filtrates	Increase in reducing power in terms of 0.05N $Na_2S_2O_3$ Solution					
		1 hr.	6 hr.	10 hr.	20 hr.	35 hr.	45 hr.
24 hr. shake culture	7.25	0.05	.....	0.30	0.85	1.50	1.95
48 hr. shake culture	7.45	Nil	.....	0.20	0.55	1.00	1.30
48 hr. stationary culture	6.60	0.25	.....	1.65	3.35	5.35	6.45
4 day shake culture	7.50	0.05	.....	0.55	1.20	2.20	2.90
4 day stationary culture	6.10	0.30	2.25	3.80	7.35	9.65	9.35
7 day shake culture	7.95	0.10	0.45	1.20	2.35	4.60	5.45
7 day shake culture – $CaCO_3$	7.60	0.05	0.10	0.25	0.65	1.35	1.70
7 day stationary culture	6.30	0.35	2.15	5.05	8.10	9.65	9.55
7 day stationary culture $CaCO_3$	6.50	0.20	1.10	2.55	4.40	6.25	6.50
10 day stationary culture	6.60	0.25	1.45	3·75	7.75	9.75	9.25
Controls		Slight decrease in reducing power					

The activities recorded for the filtrates and of the exo- and endo-cellular pectinases are represented in Table VII. It is clear therefrom that the *Xanthomonas* pectinase was active at pH 8.0 but not at pH 4.5. Further, from the calculations made for the recovery of exocellular pectinase, it would appear that only about 80 % of it was obtained by precipitation with 2 volumes of acetone at pH 6.75. This low recovery prompted us to ascertain whether certain co-factors were eliminated during the precipitation. Items 7 and 8 in the table show that neither addition of steamed culture filtrate nor that of 0.05 % yeast-extract to the reaction mixture enhanced pectinase activity.

Because of the low recovery of exocellular pectinase with 2 vols of acetone it was considered worthwhile to precipitate the exocellular pectinase at 90% saturation of $(NH_4)_2SO_4$. This gave yields of 5 – 10%. The pectinase activity of the culture filtrate and the exo- and endo-cellular preparations of one typical experiment are recorded in Table VIII. *Xanthomonas* pectinase was poor in its action on polygalacturonate as a substrate. However, a slight enhancement of activity of exocellular pectinase on polygalacturonate was attainable in the presence of 0.001 M $CaCl_2$ and a marked effect of Ca^{++} was evidenced at 0.0005 M. (Table IX). Though Ca^{++} activation was observed in these experiments further work with properly dialysed preparations of the "endo" and "exo" enzymes only could be expected to yield clear-cut results.

TABLE VII

Polygalacturonase activity of *Xanthomonas* sp. (No. 110)

	50 ml. 1.0 %polygalacturonic acid substrate pH 8.0 plus	Increase in reducing power in terms of ml. of 0.05 N $Na_2S_2O_3$ solution				
		1 hr.	5 hr.	10 hr.	20 hr.	45 hr.
1.	50 ml. culture filtrate	0.35	1.55	5.15	8.90	10.15
2.	100 mg. exo- preparation	0.20	0.80	2.95	6.00	10.20
3.	100 mg. endo- preparation	0.25	1.75	5.95	9.65	10.45
4.	50 mg. exo- preparation	0.10	.....	0.75	1.45	3.70
5.	4. above plus 0.001M $CaCl_2$	0.10	.....	0.90	1.80	4.15
6.	4. above at pH 4.5	Nil	.....	Nil	Nil	Nil
	Controls	Slight decrease in reducing power in controls for 1 and 3				
7.	50 mg. exo- preparation dissolved in steamed culture filtrate	0.10	0.35	0.70	1.55	3.70
8.	50 mg. exo- preparation plus 0.05 % yeast-extract	0.10	0.25	0.55	1.20	2.85
	Controls	No change				

TABLE VIII

Polygalacturonase activity cf the culture filtrate EXO-and ENDO-cellular preparations* using *Xanthomonas* sp. (No. 110)

50 ml. 10% polygalacturonic acid substrate pH 8.0 plus	Increase in reducing power in terms of ml. of 0.05N $Na_2S_2O_3$ solution								
	1 hr.	4 hr.	8 hr.	18 hr.	31 hr.	42 hr.	55 hr.	70 hr.	pH †
50 ml. culture filtrate	0.25	1.80	4.40	9.30	10.30	9.90	10.0	9.80	7.0
100 mg. endo-preparation	0.95	1.75	4.30	9.40	10.45	10.35	10.55	10.35	7.4
100 mg. exo-preparation	0.15	0.40	0.70	1.90	3.75	5.00	6.55	7.60	7.2
50 mg. exo-preparation	0.05	0.20	0.20	0.70	1.40	2.05	2.95	3.80	7.35
50 mg. exo-prepartion + 0.001M $CaCl_2$	Nil	0.20	0.40	1.10	2.20	3.10	4.20	5.20	7.35
50 ml. 1% pectin + 50 mg. exo-preparation	0.10	0.15	0.25	0.40	0.75	0.85	4.20	1.30	7.1
Controls	Slight decrease in reducing power								

* The enzyme preparation was obtained by precipitation with ammonium sulphate (90% saturation).

† pH of reaction mixture recorded at the end of 96 hours.

TABLE IX

Pectin and and polygalacturonic acid as substrates for *Xanthomonas* sp. (No. 110) exopectinase and the effect of Ca^{++} ions on the enzymic activity

50 ml. 1.0% polygalacturonic acid substrate pH 8.0 plus	Increase in reducing power in terms of ml. of 0.05N $Na_2S_2O_3$ solution						pH.*
	2 hr.	4 hr.	8 hr.	21 hr.	33 hr.	45 hr.	
50 ml. culture filtrate	0.70	1.80	4.50	10.0	10.15	9.90	7.3
100 mg. exo-preparation	0.45	1.10	2.70	8.00	9.85	9.80	7.45
100 mg. exo-preparation plus 0.0005 M $CaCl_2$	0.85	2.10	5.00	9.75	9.75	9.60	7.45
50 ml. 1% pectin plus 100 mg. exo-preparation	0.10	0.15	0.30	0.55	0.65	0.70	7.45
Controls	No change						

* pH of reaction mixture recorded at the end of 48 hours.

In the experiments described above buffers were not made use of for studying the reducing power of pectinases and it was considered worthwhile to find out whether buffers would influence in any way this enzyme activity. The effect of several buffers,[23] *viz.*, Tris-HCL, Phosphate and Veronal was therefore studied and the results are presented in Table X. Both Phosphate and Tris-HCl buffers were found to be unsuitable for this reaction; in fact there were indications in case of Tris-HCl buffers were found to be unsuitable for this reaction; in fact there were indications in case of Tris-HCl buffers that the cell extract might be acting on Tris itself. It would be interesting to find this out.

It was also observed during these studies that EDTA (1.0 ml of 0.1 M in 100 ml reaction mixture) caused practically complete inhibition of the enzyme reaction. Several concentrations were tried to find out the exact level of EDTA required for complete inhibition. From Table XI it may be noted that 0.002 M and not 0.001 M EDTA caused complete inhibition. The difference may be attributed to the fact that enzyme used was from the "pooled" sample whereas in the earlier experiments individual preparations were used. It is possible that the "pooled" sample may have had a higher Ca^{++} content than the individual preparations.

TABLE X

Effect of buffers on *Xanthomonas* polygalacturonase

Flask No.	pH at the end of 22 hr.	Increase in reducing power in terms of 0.05N $Na_2S_2O_3$ solution						
		1 hr.	2 hr.	4 hr.	5 hr.	6 hr.	10.5 hr.	21 hr.
1	7.1	1.55	4.55	10.55	12.15	12.60	12.65	12.60
2	5.0	2.15	5.80	10.30	9.90	9.25	8.60	8.35
3	7.8	0.50	1.40	3.90	5.25	6.75	10.65	11.90
4	8.0	4.15	7.10	9.40	12.05	12.70	12.90	12.85
5	8.05	Nil	.....	.....	Nil	0.05	0.05	0.10
6	7.8	Nil	.....	.....	Nil	Nil	Nil	Nil
7	6.6	Nil	.....	.....	Nil	Nil	Nil	Nil

Flask No. 1—50 ml. 1 per cent polygalacturonic acid, pH 8.0+20 ml. 1 per cent enzyme, pH 8 0+30 ml distilled water, pH 8.0.

No. 2—50 ml. 1 per cent polygalacturonic acid, pH 8.0+20 ml 1 per cent enzyme, pH 8.0+30 ml tris HCl buffer, pH 8.0.

No. 3—50 ml 1 per cent polygalacturonic acid, pH 8.0+20 ml 1 per cent enzyme, pH 8.0+30 ml phosphate buffer, pH 8.0.

No. 4—50 ml. 1 per cent polygalacturonic acid, pH 8.0+20 ml 1 per cent enzyme, pH 8.0+30 ml veronal buffer, pH. 8.0.

No. 5—Same as Flask No. 2, but 1.0 ml. 0.1 M EDTA+29 ml. Tris-HCl buffer pH 8.0.

No. 6—Same as Flask No. 3, but 1.0 ml. 0.1 M EDTA+29 ml phosphate buffer, pH 8.0.

No. 7—50 ml of 1 per cent polygalacturonic acid, pH 8.0+50 ml distilled water, pH 8.0.

TABLE XI

Effect of EDTA on the *Xanthomonas* polygalacturonase

Flask containing	Increase in reducing power in terms of 0.5N $Na_2S_2O_3$ solution							
	1 hr.	1.5 hr.	2 hr.	3 hr.	4 hr.	6 hr.	11 hr.	22 hr.
0.002 M EDTA	Nil	Nil	Nil	.....	Nil	Nil	.....	Nil
0.001 M EDTA	0.20	.....	0.55	.....	1.75	3.45	10.35	12.75
0.00075 M EDTA	0.65	.....	1.85	___	5.15	9.10	12.60	12.45
0.0005 M EDTA	1.60	2.95	4.30	7.70	10.20	12.75	12.90	12.60
0.00025 M EDTA	3.10	5.60	8.00	11.30	12.40	12.95	12.75	___
Nil	3 55	6.55	9.15	11.95	12.60	12.90	12.75	.....

The chromatography of the reaction mixture of the *Xanthomonas sp.* revealed, in every experiment, two spots on chromotograms even before the chromatograms were heated for the development of colour with the reagent. One of the spots was the slow running pinkish one and the other yellow and fast running. On heating, however, both merged into each other and assumed light brown colour. The reaction mixture as well as this spot did not give any of the tests of D-galacturonic acid. In the tests made with *C. laurentii. S. viridochromogenes, C. barkeri, Erwinia, A. niger* and N. B. C. pectinase (control) D-galacturonic acid was easily detected by these tests.

From all this it would seem that xanthomonad pectinase does not give D-galacturonic acid as the final product, though it is possible that it gets converted further to other product/s possessing greater reducing power than D-galacturonic acid itself. If so, this would account for the increased reducing power observed in the preparations. However, when the enzyme was tested with D-galacturonic acid alone, no increase in the reducing power was observable nor was *trans*-eliminase detected unlike in the case of *C. barkeri. Erwina sp.* and *S. viridochromogenes*[24]. This would mean that the possibility of the production of unsaturated monomer via the unsaturated dimer from the substrate has to be ruled out and an hitherto unknown mode of attack on polygalacturonate has to be postulated. This possibility is being examined. In the meantime, it is interesting to report that these cultures were found to ferment D-galacturonic acid in a complex medium. It might be mentioned at this stage that similar cultures of *Xanthomonas* sp. were isolated from rets of *sisal* (*Agave L*) leaves and they exhibited an identical pattern of mode of attack on polygalacturonate. The cultures from *sisal*, however, failed to utilise D-galacturonio acid either in a complex peptone medium or in a synthetic medium[25]. The reasons for the anomaly is far from understood and it is hoped that further work on these cultures would provide the answers.

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