POLYGALACTURONASE ACTIVITY IN YEAST, NEUROSPORA AND TOMATO EXTRACT

by

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INTRODUCTION

In 1936 the author[9,10] reported the ability of certain yeasts to attack cell wall pectin (protopectin). Since the papers were in Dutch and therefore had a limited distribution, the main conclusions are repeated below. About half of the yeast strains isolated from fermenting cacao beans in Java were able to macerate the outer layer of juicy tissue on these beans, thus facilitating the estate practice of washing the fermented beans prior to drying. As anticipated, suspensions of such yeasts also loosened the collenchyma cells in transverse sections of plant stems. This did not occur, however, after heat treatment, proving that it is an enzyme action, "protopectinase". This sensitive microscopical method of demonstrating the enzyme seems to be little known, although it was described by SLOEP[11] as early as 1928. The suitability of collenchyma tissue is obviously due to its high protopectin content. The macerating activity of the yeasts from Java cacao was shared by yeasts isolated from West African cacao (DADE) and also from other materials. The protopectinase-producing yeasts belonged to widely different genera, *e.g.*: *Candida, Pichia, Saccharomyces, Zygosaccharomyces.*

They differ from other fungi which macerate plant tissues in the absence of pectinesterase (PE) and true polygalacturonase (PG), shown by the fact that growing the yeasts for several days on yeast extract containing 1% glucose and 2% pectin caused no substantial reduction in the quantity of material subsequently precipitated by ethanol.

Only, the precipitate was less gelatinous than beforehand, which suggested a partial depolymerization.

Pectin breakdown by yeasts was also reported by Russian authors (see 3).

Recently LUH AND PFAFF[8] confirmed the absence of PE and true PG in yeasts, but in one species of the many tested, *viz. Saccharomyces fragilis* (= *Candida pseudotropicalis*), they found an enzyme which partially hydrolysed pectin and which was similar to the enzyme "pectic-acid-depolymerase" found in tomato[4,5].

A similar "depolymerase" was reported from *Neurospora crassa*[8].

We thereupon decided to investigate the nature of the final depolymerization residue produced by these different "depolymerases", by applying the chromatographic method of JERMYN AND TOMKINS[2].

To our astonishment galacturonic acid was always produced, suggesting that "depolymerase" was not the sole pectin-hydrolysing enzyme. The fact seemed worth communicating, although so far only qualitative data have been collected. During the preparation of the manuscript a short communication of PFAFF AND LUH[7] came to our notice, and fully confirms part of our results.

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METHODS

a. *Pectin hydrolysate produced by yeasts or Neurospora*

Pure cultures obtained from the C.B.S.*, were cultivated at 30° C in vials on a 1:1 mixture of yeast extract and water, containing 1% glucose and 4% pectin, the two solutions having been sterilized separately to prevent a Maillard reaction. Both high methoxyl *Citrus* pectin, l.m. apple pectin and sodium pectate from apple pectin were used. After 2, 3 or 5 weeks growth, samples were taken, preserved with toluene and stored in a refrigerator.

b. *Pectin hydrolysate produced by tomato extract*

Ripe tomatoes were pulped and then the pH adjusted to 7.5. The pulp was pressed through cloth and the sugars present fermented with 1 g baker's yeast per 10 ml at 35° C for 3 h. The solid matter was centrifuged off and the residual liquid stored with toluene for one night. The liquid was then centrifuged again and, if necessary, the pH was adjusted to 4.5. Subsequently 2% pectin or pectate and some toluene were added. Samples were taken after one and two weeks incubation at 30° C.

c. *Paper chromatography*

The hydrolysates were mixed with an equal volume of ethanol to precipitate pectin, centrifuged and then applied in eight spots near the centre of a circular Whatman no 1 filter paper[1]. The paper was irrigated centrifugally at 20° C for 24 h with a mixture of 300 ml *iso*butyric acid (fraction distillating at 154–156° C) and 205 ml water, as used by JERMYN AND TOMKINS[2]. After drying in hot air the paper was sprayed with a mixture of 1.65 g trichloroacetic acid and 1.82 g benzidine in 100 ml 96% ethanol and then kept at 105° C for 5 min. The precipitation with ethanol improved the chromatographic separation, but did not reduce the number of rings obtained.

As a reference a chromatogram was run with a pectin hydrolysate, which had been prepared with the commercial mould enzyme preparation Pectasin W, the depolymerization having been stopped so that five rings were obtained. These corresponded presumably to mono- to penta-galacturonic acid[2].

For separation of pentoses running with butanol-acetic acid, water was preferred with aniline phthalate[6] as a developing spray.

d. *Demonstration of tissue macerating enzyme*

Transverse microtome sections of stems of *Lamium album* were kept for two days at 25° C in small stoppered tubes with culture solutions or tomato extract provided with thymol. Then the sections were compared microscopically with controls kept in heated solutions.

RESULTS

Results are presented in Table I. The yeasts, the tomato extract and the mould enzyme all produced pentose from pectate and the pectins; pentose was obviously metabolised by *Neurospora*. Sometimes two rings due to pentose were visible, but this phenomenon received no further attention.

The depolymerization with mould enzyme was purposely stopped at the stage producing the five galacturonide rings so that it could be used as reference, but no ethanol-precipitable pectin was then left. Of course *Lamium* sections were macerated heavily.

The *Neurospora* hydrolysates collected after two and three weeks showed the mono- to tetra-galacturonide rings, but the penta-polymer might have been present at an earlier stage. After five weeks only traces of the di- and tri-polymers were left in addition to much mono-galacturonic acid. There was a small quantity of ethanol precipitate and a marked macerating effect.

Most hydrolysates produced by yeasts revealed three or four rings, presumably mono- to tetra-galacturonic acid. Less intense rings were correlated with a greater quantity of ethanol precipitate and with a less marked macerating effect. In several hydrolysates only traces of galacturonic acid and of its polymers were found. Then a heavy or even a gelatinous ethanol precipitate was observed and no macerating effect at all. Pentose, however, was always produced. There was no difference between the

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TABLE I

		C.B.S. culture nr	Pentoses	Galacturonide					Ethanol precip.*	Lamium test
				mono	di(?)	tri(?)	tetra(?)	penta(?)		
Neurospora crassa + and —			—	+	+	+	+	—	t	+
Pektasin W enzyme (correct duration)			+	+	+	+	+	+	none	++
Tomato enzyme			+	+	+	+	—	—	m	+
Candida pseudotropicalis	(Cast.) Basgal	35–6–1	+	+	+	+	+	—	m	+
Candida pseudotropicalis	strain *Torula cremoris*	35–6–3	+	+	+	+	+	—	h	+
Candida pseudotropicalis	var. *lactosa* (Harrison) Diddens et Lodder	35–6A–1	+	+	+	+	+	—	s	+
Candida krusei	(Cast.) Berkhout, from W.A. cacao	35–3–3	+	±	±	±	±	—	gel.	—
Candida utilis	(Henneberg) Lodder et v. Rij. = *Torulopsis utilis*	24–9–1	+	—	—	—	—	—	gel.	—
Candida macedoniensis	(Cast. et Chalmers) Berkhout	35–12–1	+	+	+	+	—	—	m	+
Saccharomyces marxianus	Hansen	2–17–1	+	+	+	+	±	—	m	+
Saccharomyces cerevisiae	Hansen, from Java cacao (II 15)	8–56–1	+	+	+	+	±	—	m	+
Saccharomyces cerevisiae	Hansen, strain Delft I	8–1–2	+	±	±	±	±	±	h	—
Saccharomyces cerevisiae	Hansen, isolated from baker's yeast		+	±	±	±	±	—	h	—
Saccharomyces cerevisiae	Hansen, baker's yeast autolysate		+	±	±	±	—	—	h	—
Saccharomyces cerevisiae	var. *ellipsoideus* (Hansen) Dekker, from Java cacao (II 7)	8–55–1	+	+	+	+	+	—	h	+
Saccharomyces chevalieri	Guilliermond, from Java cacao (II 5)	8–54–1	+	+	+	+	±	—	h	+
Saccharomyces fragilis	Jörgensen	8–12–1	+	+	+	+	±	—	m	+
Pichia fermentans	Lodder, from Java cacao (II 1)	9–14–1	+	±	±	—	—	—	gel.	—
Pichia fermentans	Lodder, from W.A. cacao	10–11–1	+	+	+	+	—	—	m	+
Hansenula anomala	(Hansen) H. et P. Sydow	10–1–1	±	—	—	—	—	—	gel.	—
Kloeckera apiculata	(Reess emend. Klöcker) Janke, from W.A. cacao	20–12–1	+	—	—	—	—	—	gel.	—

* gel. = gelatinous; h = heavy, viscous solution; m = moderate, non viscous sol.; s = small; t = trace.

chromatograms of yeast hydrolysates collected after two and five weeks incubation. We suppose that old cultures do not produce the enzyme and that the enzyme produced earlier is destroyed in the course of time. As a rule more uronides were produced from pectate than from the pectins. There was no visible difference between the hydrolysates from l.m. apple and l.m. *Citrus* pectins.

Tomato extract also produced galacturonic acid and at least the di-and tri-polymers. The character of the ethanol precipitate indicated moderate hydrolysis and there was a macerating effect on *Lamium* sections.

CONCLUSIONS

In contrast to the current view, yeasts, tomato extract and *Neurospora* do contain true PG, and not merely a "depolymerase", producing only galacturonides from pectin. With yeasts all gradations occur from no depolymerization at all to a marked PG-activity. This is correlated with the intensity of the macerating effect in collenchyma tissue. If the presence of a "depolymerase" has to be accepted on other grounds, then in all these plants the occurrence of a small quantity of PG cannot be denied.

All the yeasts tested depolymerised the pentosan occurring in the pectin preparation. Since yeasts which do not attack pectin also produce pentose, this cannot be a product of galacturonic acid decarboxylation. *Neurospora* probably metabolises the pentose formed.

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SUMMARY

In culture solutions of several yeast species and of *Neurospora crassa* and also in tomato extract, a real but often small polygalacturonase activity has been demonstrated. It is correlated with a macerating effect on collenchyma tissue. Some yeasts lack the ability to attack pectin.

RÉSUMÉ

Nous avons démontré la présence d'une activité de polygalacturonase vraie mais souvent faible dans les cultures de plusieurs levures et de *Neurospora crassa* ainsi que dans un extrait de tomate. Cette activité est en rapport avec un effet de macération qui s'exerce sur collenchyme. Certaines levures ne sont pas capables d'attaquer la pectine.

ZUSAMMENFASSUNG

In Kulturlösungen mehreren Hefearten und von *Neurospora crassa*, sowie in Tomatenextrakt wurde eine echte, aber oft geringe Polygalakturonaseaktivität nachgewiesen. Sie steht in Wechselbeziehung mit einem Mazeration von Kollenchymgewebe. Einigen Hefen fehlt die Fähigkeit Pektin anzugreifen.

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