BIOLOGIA PLANTARUM (PRAHA)

10 (4) : 245—250, 1968

Pectic Enzyme Production and Morphogenesis in *Helminthosporium atypicum*

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Received September 25, 1967

Abstract. The production of pectic enzymes by *Helminthosporium atypicum* and its morphogenesis on different media were studied. It was observed that the fungus produces pectic enzyme (macerating enzyme) adaptively. Increasing concentrations of glucose had an inhibitory effect on enzyme production. Glucose promotes profuse growth and early sporulation whereas presence of pectin slows down the growth and delays sporulation. Delay in sporulation is the effect of presence of pectin and not of the low pH of the medium. It is also suggested that in the case of *H. atypicum* low pH of the medium does not allow the fungus to utilize a carbon source as efficiently as at higher pH.

The genus *Helminthosporium* LINK includes several phytopathogenic species mostly parasitizing cereal crops and other graminaceous hosts. As the diseases caused by these species like leaf-spot, root rot, footrot and netblotch are of considerable economic importance, it is not surprising that they and and their causal orgaisms have been extensively studied. The studies of the last six to seven decades are mostly devoted to the symptomology and etiology of diseases, host range of these species and control measures. The literature hitherto reveals that the species belonging to this genus have not been studied for their enzyme-equipment as well as for morphogenesis. Therefore this paper deals with the production of macerating enzyme (protopectinase) by a species of *Helminthosporium, H. atypicum*, and its morphogenesis.

Materials and Methods

Four different media with the following composition were used to study the production of macerating enzyme and to follow morphogenesis of the fungus.

I. Glucose nitrate medium (GN): Glucose 1%, KNO_3 0·25%, KH_2PO_4 0·1% and $MgSO_4 . 7H_2O$ 0·05%.

II. Glucose nitrate + 1 % pectin (GN + P).
III. Pectin nitrate (PN) in which glucose in (1) was replaced by an equivalent quantity of pectin.
IV. Pectin peptone medium (PP): pectin 1%, peptone 0·25%, KH_2PO_4 0·1% and $MgSO_4 . 7H_2O$ 0·05%.

25 ml of the medium were distributed in 250 ml conical flasks and after autoclaving in the usual way were inoculated in triplicate with 0·5 ml of spore suspension prepared by adding 10 ml sterile water to eight day old PDA slant culture of the fungus. The flasks were incubated at 26 ± 3° C for eight days. Changes in pH were determined with a pH meter and the dry weight of mycelial mat was found out by transferring it onto previously weighed Whatman No. 1 filter paper and drying it at 60° C for 24 hours.

Macerating activity was estimated by the BROWN (1915) method. Cylindrical plugs, 8 mm in diameter were cut from healthy potato tubers with a No. 4 cork borer. The plugs were injected with tap water under vacuum for one hour. Discs 0·4 mm thick were cut with a sliding hand Microtome from these plugs. They were washed quickly with tap water and then distilled water and stored in distilled water in a Petri dish. Five discs were placed in three ml of an enzyme solution in a watch glass. At intervals of 5 minutes these were subjected to slight tension by hand, and as soon as the first disc had lost coherence, tests were continued till all the discs lost coherence. The mean time for loss of coherence was noted and taken as the reaction time (R.T.) in minutes.

To study the morphogenetic changes during growth for 8 days, slides of the cultures were prepared on the third, 6th and 8th day of incubation and macroscopic and microscopic observations were recorded.

Results and Discussion

1. Growth and production of macerating enzyme

It has been observed by many authors (BROWN 1915, WOOD 1955, SADASIVAN and SUBRAMANIAN 1963) that fungi produce pectic enzymes in a medium with or without pectin. DESHPANDE (1960) has pointed out that peptone is more suitable as a nitrogen source for obtaining powerful macerating

Table 1
Production of macerating enzyme by *Helminthosporium atypicum*

Medium	Initial pH	Final pH	Mean dry wt (mg)	R. T
GN	5·5	7·3	120	no activity
GN + P	2 8	3·1	145	20 hours
PN	2·5	2·8	55	8 hours
PP	3·1	3·1	65	22 minutes

enzyme. In order to find out the adaptive or constitutive nature of the macerating enzyme produced by *H. atypicum*, and suitability of peptone as a nitrogen source, four media were inoculated and culture filtrates collected on the 8th day were tested for macerating activity (Table 1).

From the data it is clear that the fungus was unable to secrete the macerating enzyme in the absence of pectin and therefore production of macerating enzyme is adaptive. Comparison between the activities of filtrates from GN + P an PN further indicated that glucose acted as inhibitor to the

Table 2

Effect of various concentrations of glucose upon production of macerating enzyme by *Helminthosporium atypicum*

Glucose %	Initial pH	Final pH	Mean dry wt (mg)	R. T. (min)
0·1	3·4	2·8	45	30
0·25	3·4	2·8	45	35
0·75	4·0	3·1	50	45
1·0	4·0	3·1	80	50
Control (PP medium)	3·1	3·1	65	22

production of macerating enzyme. Moreover, when results of macerating activity of filtrates from PP and PN medium were compared, peptone was found to be a better nitrogen source than KNO_3.

In order to find out whether glucose had a similar inhibitory effect at low concentrations it was added to PP medium in different quantities. Filtrates were collected after 8 days and tested for macerating activity (Table 2).

Results given in Table 2 indicate that there is a fall in pH during growth, an increase in growth with increased concentrations of glucose and a reduction in macerating activity with larger quantities of glucose. It is clear, therefore that glucose at higher concentrations is inhibitory to the enzyme production.

2. Growth and morphogenesis (Table 3)

The morphogenetic changes of the fungus during its growth in these four media were also followed on the 3rd, 6th and 8th day. In GN medium with initial pH 5·5, the fungus grew fairly well, formed a semi-transparent olivaceous mat and sporulated on the 3rd day. Spores were pale yellow to brown and 0—7 septate. The mycelial mat thickened on the 6th day and became dark olivaceous. One or two compact, spongy, white and raised stalked pseudoclerotia appeared on the mat. The spores were dark brown and 0—10 septate. Atypical spores increased in number (70%). On the 8th day the mat was thick, black; pseudosclerotia increased in number covering half of the surface of the mat; spores were dark brown, fully mature and in some spores thick walls obscured the septation; pH showed a definite drift towards alkalinity.

In the case of GN + P medium with initial pH 2·8, colony was in the form of yellow specks floating in the medium and showing spore germination and

Table 3
pH and morphogenesis of *Helminthosporium atypicum*

Medium	3rd day		6th day		8th day	
	pH	morphogenesis	pH	morphogenesis	pH	morphogenesis
GN	6·4	Mycelial mat thin, olivaceous. Sporulation initiated. Young spores 0-7 septate, brown.	7·0	Mycelial mat olivaceous black. Spores 0-10 septate, dark brown. Pseudosclerotia appear in the medium.	7.3	Mat thick pseudosclerotia increase in number. Septation in spores obscured.
GN + P	2·8	Colony in the form of yellow specks. Spores germinating, germ tubes branched.	3·1	Colony in the form of yellow patches 2-3 cm in diameter, with central olivaceous raised part. Vegetative growth only.	3·1	Colony in the form of almost continuous olivaceous mat. Sporulation started. Young spores 0-6 septate, pale brown.
PN	2·5	Colony as minute hyaline specks, spore germination initiated.	2·8	Colony as pinhead-sized yellow.specks. Germ tubes slightly elongated, brown, branched.	3.1	Colony in the form of yellow patches 1-3 mm in diameter. Only branching of germ tubes.
PP	3·1	—do—	3·1	—do—	3·1	—do—

branching of germ tubes on the 3rd day. Germ tubes and their branches were filled with granular contents and were beaded. On the sixth day these specks developed into large yellow patches about 2—3 cm in diameter; these patches showed raised olivaceous growth at their centres and it was only vegetative growth. The mat was thick and almost continuous on the 8th day. It had olivaceous to black colour and sporulation could be observed, young spores were pale brown and 0—6 septate. The pH remained approximately the same.

The fungus exhibited a similar pattern of growth on PN and PP media. Colony was in the form of minute, hyaline specks on the third day. These

Table 4
Effect of pH on sporulation in GN and PN medium

Medium	Initial pH	pH at the time of sporulation	Dry wt* (mg)	Day of sporulation
GN	3·1	6·4	50	10
—do—	5·5	6·4	90	3
PN	2·5	5·8	85	20
—do—	5·5	5·8	80	16

* Dry weight here corresponds to the day of sporulation respectively.

were composed of germ tubes only. On the 6th and 8th day these specks developed into slightly larger yellow patches. Germ tubes showed branching on the 6th day and on the 8th day also only vegetative growth appeared. There was a slight increase in pH in PN medium, and no change in pH in PP medium.

It is obvious that in GN, growth was fast and sporulation was abundant which indicated that the presence of glucose was essential for growth and sporulation. Growth in PN and PP (Table 1) when compared to that in GN + pectin is very poor, which further proved that in spite of similar initial pH in all these three media only the absence of glucose in PN and PP medium resulted in poor growth. Moreover, pectin appears to be responsible for delay in sporulation. To confirm whether delay in sporulation was the effect of low pH or of pectin, another experiment was conducted and the results are given in Table 4.

These results further confirm that pectin, when present in the medium, delays sporulation. Pectin is also responsible for lowering the pH of the medium. But in spite of low pH pectin allowed sporulation in combination with glucose (Table 3). Therefore it can be stated that delay in sporulation is the direct effect of pectin as the only carbon source in the medium.

In addition, results given in Table 4 also indicate that low pH of the medium does not appear to allow quick utilization of glucose or pectin; consequently, growth slows down and sporulation is delayed. On the contrary higher pH enables the fungus to utilize the carbon source quickly; there is increase in growth and early sporulation.

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K. S. DESHPANDE, K. B. DESHPANDE, Botanické oddělení university Marathwada, Aurangabad, India: **Tvorba pektického enzymu a morfogenese u *Helminthosporium atypicum*.** - Biol. Plant. **10** : 245—250, 1968.

Byla studována morfogenese a tvorba pektického enzymu u *Helmintosporium atypicum*. Bylo zjištěno, že houba vytváří pektický (macerační) enzym adaptivně Zvýšená koncentrace glukosy potlačovala tvorbu enzymu. Glukosa stimulovala růst a urychlovala sporulaci, zatímco přítomnost pektinu růst brzdila a zpožďovala sporulaci. Zpoždění sporulace je způsobeno přítomností pektinu a nikoliv nízkým pH média, předpokládá se také, že u *H. atypicum* snížení pH media snižuje využití zdrojů uhlíku.

Деспанд, К. С., К. Б. Деспанд, Ботанический отдел университета Маратвада, Аурангабад, Индия **Продукция пектиназы и морфогенез у *Helminthosporium atypicum*** — Biol. Plant. **10** . 245—250, 1968.

Изучалась продукция пектиназы и морфогенез у *Helminthosporium atypicum* выращиваемого на культивационных средах разного состава. Установлено, что продукция пектиназы (мацерирующий фермент) носит адаптивный характер. Повышение концентрации глюкозы действует ингибирующе на продукцию фермента. Глюкоза значительно усиляет рост и приближает срок споруляции, в то время как пектин снижает рост и передвигает фазу спорулирования на более позднее сроки Задержка споруляции является следствием добавления пектина а не низкого pH культивационной среды. Низкое pH среды по видимому снижает способность *H atypicum* эффективно использовать источники углерода