ACTA MYCOLOGICA
vol. XXIV(2) :185 – 192
1988 (1989)

Biochemical changes in the mycelium of two Rhizoctonia solani isolates during autolysis

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R e d d y M. N.: *Biochemical changes in the mycelium of two Rhizoctonia solani isolates during autolysis*, Acta Mycol. 24(2): 185 – 192, 1988 (1989).

Some biochemical changes occurring in the mycelium of two isolates (one pathogenic FR and another non-pathogenic GD2) of *R. solani* during autolytic phase of their growth were studied, by growing the fungi for periods longer than 210 days. During autolysis a decrease of 76,4% and 78,5% in mycelial dry weight occurred in pathogenic and non-pathogenic isolates respectively, compared to that at the beginning of autolysis. The mycelium of non-pathogenic isolate was more affected during autolytic phase of growth than that of the pathogenic isolate.

INTRODUCTION

Recently the study on chemical changes in the autolytic phase of fungal growth have been the subject of interest. The nature and variation in the amount of various compounds have attracted the attention of many workers. Much of our knowledge on the chemistry of autolysis in filamentous fungi came from the research work done on species of the genera *Aspergillus, Nectria, Sclerotinia, Penicillium* and *Sclerotium* and *Rhizoctonia*. Though the previous studies have delineated some of the biochemical changes that occurred in *Rhizoctonia solani* Kuhn with increasing age of mycelium (G o t t l i e b, Van E t t e n 1966; N i c o l s, G o t t l i e b 1968; O b r i g, G o t t l i e b 1970), the work reported here was undertaken as a further contribution to this aspect of chemistry of aging. The behaviour of certain intracellular components (nitro-genous and non-nitrogenous) in the mycelium of two isolates of *R. solani*, during autolysis were studied.

MATERIAL

The study concerned the some two isolates of *R. solani*, *Arachis hypogaea* L. var TMV2 rhizosphere (pathogenic FR and non-pathogenic GD2), that already

used for various biochemical and physiological work in the laboratory (Reddy, Rao 1975; Reddy 1876).

METHODS

The isolates were grown on Chapek-Dox liquid medium with 3% sucrose at Ph6,8 in 200 ml aliquots in 1 l Roux bottles. After autoclaving each flask was inoculated with a 5 mm mycelial disk (from the periphery of a two-days'old culture plate) and incubated for longer than 210 days. At intervals flasks were randomised and sample flaske were taken for analysis. The mycelium was separated from the culture fluid by filtration and washed several times with distilled water, blotted dry and used for extraction as described below. Parallel samples were used for dry weight.

The mycelial mat was chopped small pieces and 1 g sample of the material was transferred to about 25 ml of boiling 80% ethanol, extracted for 10 min on a hot water bath by refluxing and then cooled. The material was homogenized through wet cheese cloth. The residue was transferred back to a small quantity of fresh boiling 80% ethanol and reextracted for 5 min. Both the extracts were pooled and centrifuged. The supernatant was concentrated and made up to 5 ml.

1 ml aliquots of the extract were used for the estimation of orto-dihydric phenols (Johnson, Schaal 1957), total phenols (Bray, Thorpe 1954), reducing sugars (Nelson 1944), non-reducing sugars (Inman 1965) and amino nitrogen (Moore, Stein 1948).

The method of the mycelium extraction for free and bound amino acids, their asparation by 2-dimentional paper chromatography and their identification were the same as in Reddy, Rao (1975).

The mycelial mats were weighed after drying in a hotair oven at 80C for 24 hours. The values reported were the averages of at least three replicates.

RESULTS

By using the loss of mycelial dry weight as a criterion, autolysis began at 15-16 days of incubation. The 16th day of incubation was therefore taken as zero day of autolysis. During autolysis a loss of 76,4% and 78,5% in mycelial dry weight was observed for the isolates FR and GD2, respectively compared their weight on the zero day (Table 1).

In both isolates the content of total phenols, orthodihydricphenols (OD phenols) and amino nitrogen increased during the first days of autolysis with a subsequent decrease thereafter. On the other hand, reducing and non-reducing sugars decreased steadily throughout the period of autolysis. The loss of various components due to analysis amounted to 83,7% – total phenols, 66,7% – OD phenols, 64,4% – amino nitrogen, 95,1% – reducing sugars and 85,3% – non

Table 1

Variation in dry weight of mycelium and content of total phenols, orthodihydric phenols, amino nitrogen and reducing and non-reducing sugars in the mycelium of two isolates (pathogenic FR and non-pathogenic GD2) of *R. solani* during autolytic phase of growth.

Incubation time – days	Period of autolysis (days)	Mycelium dry Wt. g/flask		Total phenols µg/g fresh Wt.		Ortodihydric phenols µg/g fresh Wt.		Amino nitrogen µg/g fresh Wt.		Reducing sugars µg/g fresh Wt.		Nonreducing sugars µg/g fresh Wt.	
		FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2
15	0	4.05	2.80	56	125	30	12	225	200	0.615	0.675	1.225	1.325
16	1	3.95	2.75	59	127	35	14	227	205				
30	15	3.13	2.20	70	132	60	20	240	260	0.49	0.51	0.84	1.00
60	45	2.85	1.89	72	88	62	25	276	250	0.36	0.43	0.60	0.80
90	75	2.56	1.27	60	80	35	16	220	185	0.27	0.32	0.52	0.59
120	105	2.28	0.87	26	20	30	10	186	140	0.12	0.20	0.40	0.48
150	135	1.67	0.70	20	18	21	8	152	96	0.07	0.17	0.28	0.34
180	165	1.14	0.67	13	14	14	6	98	75	0.04	0.09	0.20	0.29
210	195	0.95	0.60	9	8	10	4	80	60	0.03	0.07	0.18	0.22

Table 2

Free amino acids in the autolysing mycelium of two isolates (pathogenic FR and nonpathogenic GD2) of *R. solani*

Amino acid	Period of autolysis (days)																	
	0		1		15		45		75		105		135		165		195	
	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2
Cystine	+	0	+	0	+	0	+	0	+	0	+	0	+	0	T	0	–	0
Aspartic acid	+	+	+	+	+	+	+	+	+	+	+	+	T	T	T	T	–	T
Glutamic acid	+	+	+	+	+	+	+	+	+	+	+	T	T	T	–	T	–	T
Serine	+	+	+	+	+	+	+	+	+	+	+	T	T	–	T	–	–	–
Glycine	+	+	+	+	+	+	+	T	+	–	+	–	T	–	T	–	–	–
Lysine	+	+	+	+	+	+	T	T	–	–	–	–	–	–	–	–	–	–
Histidine	+	+	+	+	+	+	+	+	+	T	T	–	–	–	–	–	–	–
Glutamine	+	+	+	+	+	+	+	+	+	T	+	T	–	–	–	–	–	–
Threonine	0	+	0	+	0	+	0	T	0	–	0	–	0	–	0	–	0	–
Alanine	+	+	+	+	+	+	+	+	+	+	+	T	+	–	T	–	T	–
Tyrosine	+	+	+	+	+	+	+	T	T	–	–	–	–	–	–	–	–	–
Tryptophan	+	0	+	0	+	0	+	0	T	0	–	0	–	0	–	0	–	0
Phenylalanine	+	0	+	0	+	0	+	0	+	0	+	0	T	0	–	0	–	0

0 absent, + present in considerable amounts, T present in traces only, – gradual disappearance.

Table 3

Bound amino acids present in the autolysing mycelium of two isolates (pathogenic FR and non pathogenic GD2) of *R. solani*

Amino acid	Period of autolysis (days)																	
	0		1		15		45		75		105		135		165		195	
	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2	FR	GD2
Cystine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	T	+	T
Cystein	+	+	+	+	+	+	+	+	+	+	+	+	T	+	T	+	T	+
Aspartic acid	+	+	+	+	+	+	+	+	T	+	T	T	T	T	–	T	–	T
Glutamic acid	+	+	+	+	+	+	+	+	+	+	+	T	T	T	T	T	T	T
Unidentified	+	+	+	+	+	+	+	+	+	+	+	T	T	T	–	–	–	–
Unidentified	+	+	+	+	+	+	+	+	+	T	+	–	T	–	T	–	T	–
Unidentified	+	+	+	+	+	+	+	+	T	+	T	T	T	–	–	–	–	–
Unidentified	+	0	+	0	+	0	+	0	T	0	–	0	–	0	–	0	–	0
Serine	+	+	+	+	+	+	+	+	+	T	+	T	+	T	T	T	T	T
Glycine	+	+	+	+	+	+	+	+	+	+	T	T	T	T	T	–	T	–
Threonine	+	+	+	+	+	+	+	+	+	T	+	T	T	T	T	–	T	–
Histidine	+	+	+	+	+	+	+	T	+	T	T	T	T	T	T	–	T	–
Glutamine	+	+	+	+	+	+	+	+	+	T	T	T	T	T	–	T	–	T
Unidentified	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alanine	+	+	+	+	+	+	+	+	+	T	T	T	T	T	T	–	T	–
Tyrosine	+	+	+	+	+	+	+	T	+	T	+	T	T	–	T	–	–	–
Proline	+	+	+	+	+	+	+	T	+	T	+	T	T	–	T	–	–	–
Tryptophan	+	+	+	+	+	+	+	+	+	T	+	T	T	T	T	–	–	–
Methionine	+	+	+	+	+	+	+	+	+	T	T	T	T	–	T	–	–	–
Unidentified	+	0	+	0	+	0	+	0	T	0	T	0	–	0	–	0	–	0
Valine	+	+	+	+	+	+	+	+	+	T	T	T	T	T	T	T	–	T
Phenylalanine	+	+	+	+	+	+	+	+	T	+	T	T	T	T	T	–	T	–
Leucine/isoleucine	+	+	+	+	+	+	+	+	+	T	T	–	T	–	–	–	–	–
Unidentified	0	+	0	+	0	+	0	+	0	T	0	T	0	–	0	–	–	–

0 absent, + present in considerable amounts, T present in traces only, – gradual disappearance.

reducing sugars for the isolate FR and 93,6% total phenols, 66,7% – OD phenols, 70% – amino nitrogen, 89,6% – reducing sugars and 83,4% – non reducing sugars, for the isolate GD2, compared their content on the zero day (Table 1).

All free amino acids of the mycelium reported for the isolates (R e d d y, R a o 1975) remained noticeable up to 45 days of autolysis. A general picture was that of their decreasing concentration (as noticed by relative intensity of the individual spots) during autolysis, eventually resulting in the disappearance of most. The concentrations of each amino acid as estimated by visual comparison are presented in Table 2.

The bound amino acids released in hydrolysis and their visual comparison are presented in Table 3. Here, there was also the general of trend of gradual decrease in the constituent amino acids. All but one unidentified compound (common to both isolates) reported (R e d d y, R a o 1975) disappeared gradually during autolysis.

DISCUSSION

Autolysis is a general phenomenon in most fungi when grown on limited quantities of media. As soon as the food supply is exhausted growth ceases and autolysis sets in. Various factors that affect autolysis include: the type medium of (alcaline or acid), nitrogen source, amount of carbon source, temperature, culture type i. e. statinary or submerged etc. So far there have been some attemps to study the chemistry of autolysis in cultures of filamentous fungi.

Carbohydrates present in the fungal mycelia are believed to be undergoing continuously breakdown during autolysis in culture (L a h o z 1967). T a n d o n and C h a n d r a (1962) have reported a decreased concentration of carbohydrates in the mycelium of *Colletotrichum gloeosporoides* during autolysis. Such a pattern of diminution, as autolysis preceeds has been reported for some other fungi (L a h o z, R e y e s, B e l t r a 1966; L a h o z, G o n z a l e s I b e a s 1968; L a h o z, M i r a l l e s 1970). Moreover, a continuous decrease in both reducing and non reducing sugars have been observed in both isolates of *R. solani* used.

Earlier studies on phenol content of mycelia of filamentous fungi during autolysis completely lacking grounds for comparison with the present results. Decrease in the mycelial nitrogen during autolysis has been reported for some fungi (L a h o z, R e y e s, B e l t r a 1966; L a h o z, M i r a l l e s 1970). Gradual decrease with age, in soluble amino nitrogen and protein has been observed in *R. solani* by G o t t l i e b and V a n E t t e n (1966). Though there has been an initial increase, present study also indicate a gradual decrease of amino nitrogen content during autolytic phase in both isolates.

There has been a marked and gradual reduction in the content of and also disappearance of some amino acids (both free and bound). The decrease may partially acount for the loss of amino nitrogen observed in the mycelium during autolysis. Marked changes in the bound amino acid pool indicate that mycelial proteins are also much affected by autolysis. The observed changes in the amino acid pools confirm the results reported on *Aspergillus flavus* by Pillai and Srinivasan (1956) and Lahoz, Reyes, Beltra (1966).

In general, the results concerning the two isolates of *R. solani* indicate that the chemical changes occuring in the mycelium during autolytic phase of growth are similar to those reported for other organisms. The results analysis reveals that autolysis seems to have more effect on the mycelium of non pathogenic isolate GD_2 than on that of pathogenic FR.

Thanks are due to Prof. Dr hab. Edmund Strzelczyk of Laboratory of Microbiology, Nicolaus Copernicus University, Torun for critical analysis and suggestions in the preparation of this manuscript.

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