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Protein utilization and release of extracellular proteinase by two root-rot fungi

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Abstract

Sclerotium rolfsii and *Sclerotium bataticola* could utilize protein and produce extracellular proteinase. In protein-deficient media no extracellular proteinase was detected. In protein-containing media addition of glucose stimulated enzyme synthesis; in absence of glucose addition of ammonium to the protein-media decreased enzyme activity. The rate of protein hydrolysis was higher in media contained glucose but not ammonium. The rate of glucose depletion from the culture filtrates was faster in treatments received protein with glucose or with glucose and ammonium, and addition of sulphur showed slight decrease in glucose utilization.

Introduction

In many fungi extracellular proteinase production can be repressed by low molecular mass compounds of carbon and nitrogen (Drucker 1972, Cohen 1973). Proteinases are induced when protein is present with one of the major elements namely, carbon, nitrogen and sulphur. In the presence of protein and all three alternative sources of the major elements proteinase production becomes repressed (Drucker 1972, Hanson and Marzluf 1973, Kalisz and Moore 1986). Some investigators proved the utilization of protein as a source of both C and N by mycorrhizal fungi (Bajwa and Read 1985, Spinner and Haselwandter 1985, Abuzinadah and Read 1986, Leake and Read 1991) while other investigators reported the use of protein as a source of C, N or S by some basidiomycete fungi (Kalisz *et al.* 1987).

The present work was undertaken to investigate the ability of two root-rot fungi to utilize protein and their potentiality for production of extracellular proteinase.

Materials and methods

Test organisms: The fungi used in this investigation were *Sclerotium bataticola* and *Sclerotium rolfsii* which were kindly supplied by Plant Pathology Research Institute, Agricultural Research Centre, Giza Egypt.

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Initial proteinase screening: Preliminary test for proteinase producing ability of the test fungi was made using nutrient agar plates containing 1.5 % gelatine for growth. The plates were incubated at 30 °C for 12 d and test for proteolytic activity was made every day by flooding two plates with 20 % HCl (Rao *et al.* 1970). The appearance of clear zone indicated the production of protease enzyme by the prevailing fungi.

Extracellular proteinase in liquid culture media: The fungi were cultivated on a modified medium suggested by Kalisz *et al.* (1986). It contained 1 % glucose (m/v) as C-source; 1 % insoluble casein; 30 mM ammonium chloride and 0.05 % $MgSO_4.7H_2O$. Combinations of the medium constituents were designed, in one casein was used as sole source of C, N and S. Three contained casein in combination of glucose, ammonium chloride or both. In the absence of $MgSO_4.7H_2O$, magnesium chloride was used. The last medium lacked protein and contained glucose, ammonium chloride and magnesium sulphate.

The different media were distributed among 250 cm^3 conical flasks by the rate of 100 cm^3 and sterilized. Each flask was inoculated with 5 mm disk taken from 7 d old culture of each of the test fungi and incubated at 30 °C under static conditions. Harvests were taken after 3, 6, 9 and 12 d of growth. There were 3 replicates for each treatment. At each harvest, mycelia were separated from the culture solutions by filtration of Whatman No. 1 filter paper, oven dried at 80 °C till constant mass.

Proteinase assay: Proteinase activity in the culture solutions was assayed at each harvest time using the procedure proposed by Somkuti and Bable (1968).

Protein determination: Residual protein in the culture filtrate was estimated after each incubation period using the Lowry *et al.* (1957) method. Protein concentration was determined against a casein standard.

Determination of residual glucose: Glucose remained in the culture filtrate after each harvest was assayed using a glucose assay kit (*Sigma No. 510*), interfering substances being removed by precipitation with 0.15 M $Ba(OH)_2$ and 5 % $ZnSO_4.7H_20$.

Results and discussion

No extracellular proteinase activity was observed in protein-deficient media indicating that the proteinases produced by *S. bataticola* and *S. rolfsii* are of inducible nature. In media supplemented with protein, high extracellular proteinase activity was observed after 9 d for cultures of *S. bataticola* and 12 d for *S. rolfsii*. In both cultures proteinase production was affected by addition of glucose which potentiated its activity to a considerable values (Table 1). Supplementation of the protein + glucose medium with ammonium induced a further slight increase in activity. On the other hand, in media deficient in glucose addition of ammonium to protein media decreased enzyme production. These findings are contradictory to those of Kalisz *et al.* (1987) who reported an increased proteinase activity of some basidiomycetes in presence of ammonium and absence of glucose.

Table 1. Proteinase concentration of *Sclerotium bataticola* and *Sclerotium rolfsii* grown on media with different supplementations of protein, glucose and ammonium. (Expressed as the amount of enzyme that solubilizes 1 ng(tyrosine) s^{-1}, 30 °C).

Test organism	Incubation time [d]	Supplements to the medium Protein	Protein + glucose	Protein + ammonium	Protein + glucose + ammonium
Sclerotium bataticola	3	425.5 ± 18.3	666.7 ± 66.7	553.3 ± 35.0	758.3 ± 88.3
	6	498.3 ± 38.3	1218.3 ± 30.0	678.3 ± 48.3	1338.3 ± 35.0
	9	1260.0 ± 25.0	2333.3 ± 20.0	968.3 ± 31.7	2510.0 ± 55.0
	12	1033.3 ± 70.0	2201.7 ± 46.7	786.7 ± 50.0	2303.3 ± 40.0
Sclerotium rolfsii	3	791.7 ± 53.3	923.3 ± 51.7	720.0 ± 90.0	928.3 ± 80.0
	6	1143.3 ± 56.7	1370.0 ± 46.7	1143.3 ± 53.3	1470.0 ± 86.7
	9	1560.0 ± 38.3	1916.7 ± 81.7	1471.7 ± 56.7	2061.7 ± 23.3
	12	1838.3 ± 15.0	2441.7 ± 43.3	1750.0 ± 80.0	2505.0 ± 76.7

The biomass yield showed decreased values in protein-deficient media. In protein containing media it was observed that addition of glucose and/or ammonium resulted in increasing yield to 1-fold or 1.5-fold in cultures of *S. bataticola* and *S. rolfsii*, respectively. In presence of both protein and glucose growth was enhanced by further supplementation with ammonium (Table 2). This indicates that both protein and glucose were utilized simultaneously. Similar observations have been made with white rot fungi such as *Pleurotus ostreatus* (Hiroi and Erikson 1976) and *Sporotrichum palverulentum* (Ander and Eriksson 1977).

Table 2. Biomass yield [mg(d.m.) cm^{-3}(medium)] of *Sclerotium bataticola* and *Sclerotium rolfsii* grown on media with different supplementations of protein, glucose, ammonium and sulphur.

Test organism	Incubation time [d]	Supplements to the medium Protein	Protein + glucose	Protein + ammonium	Protein + glucose + ammonium	Glucose + ammonium + sulphur
Sclerotium bataticola	3	12.4 ± 3.2	20.6 ± 3.7	18.0 ± 1.2	22.2 ± 3.5	8.0 ± 2.7
	6	28.5 ± 1.3	50.2 ± 4.8	37.4 ± 7.2	64.8 ± 4.3	16.0 ± 4.2
	9	37.8 ± 4.5	58.4 ± 5.2	50.3 ± 6.7	75.3 ± 5.0	23.1 ± 3.3
	12	34.2 ± 3.0	56.1 ± 6.8	48.5 ± 2.4	68.2 ± 1.9	20.3 ± 1.7
Sclerotium rolfsii	3	10.0 ± 6.1	27.4 ± 7.0	12.2 ± 4.3	18.0 ± 4.3	9.2 ± 1.1
	6	25.8 ± 3.4	40.0 ± 4.7	32.3 ± 3.6	44.3 ± 5.4	19.5 ± 2.6
	9	42.6 ± 5.3	54.3 ± 3.1	52.1 ± 4.1	58.4 ± 3.5	28.3 ± 4.2
	12	48.3 ± 5.1	63.2 ± 4.8	58.7 ± 2.6	70.6 ± 4.3	37.6 ± 2.0

When the concentration of residual protein was measured it was observed that about 79 % of the provided protein was utilized by *S. bataticola* at the 12th incubation day while *S. rolfsii* utilized 73 % of the provided protein at the same incubation time (Table 3). The rate of which casein was broken down by the two

fungal species was significantly affected by supplementation with glucose; the fastest rate of hydrolysis occurred in cultures supplied with glucose but not ammonium (Table 3). This simultaneous utilization of protein and glucose as C-source differ from that in other microorganisms where macromolecules are believed to be only degraded on the exhaustion of the easily metabolized carbon sources (Engelking and Seidler 1974, Wouters and Bieysman 1977). A some what slower rate of hydrolysis was observed in the absence of glucose and addition of ammonium to these cultures had no significant effect indicating that ammonium has no catabolic repressive effect on protein utilization by the two fungal species.

Table 3. Concentration of residual protein [mg(protein) cm^{-3}(medium)] left in the medium during incubation of cultures of *Sclerotium bataticola* and *Sclerotium rolfsii*.

Test organism	Incubation time [d]	Supplements to the medium: Protein	Protein + glucose	Protein + ammonium	Protein + glucose + ammonium
Sclerotium bataticola	0	903.0 ± 0.2	907.0 ± 2.4	902.7 ± 2.3	906.6 ± 3.2
	3	812.2 ± 0.5	735.2 ± 0.9	805.1 ± 1.1	801.1 ± 0.8
	6	510.5 ± 1.0	305.5 ± 1.6	500.3 ± 2.4	413.2 ± 1.5
	9	320.0 ± 0.6	122.2 ± 1.5	315.2 ± 0.9	224.0 ± 0.8
	12	102.0 ± 1.2	97.0 ± 0.2	123.0 ± 2.1	112.0 ± 0.9
Sclerotium rolfsii	0	913.2 ± 1.9	892.0 ± 2.4	921.2 ± 0.8	908.5 ± 2.3
	3	821.0 ± 3.1	712.4 ± 1.9	802.0 ± 1.8	800.0 ± 0.7
	6	602.5 ± 2.3	521.6 ± 1.5	594.3 ± 2.1	603.4 ± 1.5
	9	311.2 ± 1.3	209.2 ± 0.8	301.0 ± 0.9	298.4 ± 0.8
	12	196.0 ± 0.8	105.5 ± 0.8	137.3 ± 1.7	197.2 ± 1.4

Table 4. Concentration of residual glucose [mg(glucose) cm^{-3}(medium)] left in the medium during incubation of cultures of *Sclerotium bataticola* and *Sclerotium rolfsii*.

Test organism	Incubation time [d]	Supplements to the medium: Protein + glucose	Protein + glucose + ammonium	Glucose + ammonium + sulphur
Sclerotium bataticola	0	952.6 ± 6.6	948.6 ± 9.4	963.2 ± 5.4
	3	415.4 ± 4.2	648.0 ± 12.3	723.4 ± 4.7
	6	69.6 ± 4.1	293.9 ± 9.5	320.7 ± 3.0
	9	32.0 ± 3.4	198.7 ± 6.4	220.4 ± 5.8
	12	9.0 ± 4.4	74.3 ± 3.4	102.5 ± 1.4
Sclerotium rolfsii	0	949.9 ± 7.3	952.3 ± 5.9	962.3 ± 8.7
	3	520.4 ± 7.3	640.2 ± 6.1	632.5 ± 10.2
	6	154.5 ± 8.1	287.0 ± 4.4	273.8 ± 4.6
	9	53.4 ± 2.1	182.4 ± 5.2	215.6 ± 5.8
	12	14.4 ± 1.5	92.1 ± 1.0	95.4 ± 3.1

When residual glucose was measured in the culture filtrates (Table 4) it was detected that significant depletion of glucose from the culture medium had occurred by 6 d after inoculation of *S. bataticola* and 9 d for cultures of *S. rolfsii*. The rate of depletion was faster in the treatments that had received protein with glucose alone or glucose + ammonium. Addition of sulphur showed a slight decrease in glucose utilization by the two cultures.

It could be concluded that the two root-rot fungi studied produce proteinase in the presence of protein, and that presence of glucose and/or ammonium influence the enzyme activity. This fact may affect the pathogenicity of the two fungi.

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