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Changes in Fungi with Age

III. Incorporation of Amino Acids into Cells of *Rhizoctonia solani* and *Sclerotium bataticola*

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Summary. The uptake and incorporation of L-phenylalanine and L-leucine into various cell fractions of *Rhizoctonia solani* and *Sclerotium bataticola* decreased with cell age. As would be expected, most of the radioactivity from the amino acids was distributed in the soluble and protein fractions. Ratios of the radioactivity in the soluble fraction to that in the protein fraction indicated that in *R. solani* the mechanism for protein synthesis decreased with age. However, in *S. bataticola*, the decrease in protein synthesis with age may be due to a decrease in permeability to the amino acids.

Previous papers in this series have reported on the effect of cell age on the chemical composition and the respiratory enzymes of *R. solani* and *S. bataticola* (GOTTLIEB and VAN ETTEN, 1966; VAN ETTEN *et al.*, 1966). On a dry weight basis, the percentage of DNA, RNA, soluble amino nitrogen, and protein decreased with age. However, only slight changes were observed if the values were calculated on a DNA or per cell basis.

Such decreases with age in the content of soluble amino nitrogen and protein per unit dry weight are quite common in fungi (BENT and MORTON, 1964; DAWSON, 1965; GOTTLIEB and VAN ETTEN, 1964; KRISHNAN *et al.*, 1957; MEYERS and KNIGHT, 1961; PILLAI and SRINIVASAN, 1956). The decrease in protein with cell age can result from changes in the active uptake or permeability of the cell membrane to amino acids, rate of protein synthesis, or the rate of protein breakdown.

The purpose of this investigation was to determine whether the decrease in the content of soluble amino nitrogen and protein with age in *S. bataticola* and *R. solani* was caused by a reduced amino acid uptake or a reduction in protein synthesis. This was accomplished by measuring the age dependent incorporation of radioactive amino acids into the soluble and protein fractions of whole cells of these two fungi.

Materials and Methods

R. solani Kuhn and *S. bataticola* Taub. were grown, harvested, and separated into various age groups as described previously (GOTTLIEB and VAN ETTEN, 1966). Whole cell suspensions were prepared by homogenizing two g of mycelium (wet wt.)

in 20 ml of sterilized growth medium in a Waring Blendor at 4°C for 20 sec. Separate aliquots of the mycelium were taken for dry weight and protein determinations (LOWRY *et al.*, 1951). The cell suspensions were placed in a 250 ml Erlenmeyer flask, together with an additional 30 ml of growth medium. After allowing the suspension to come to room temperature, 1 μc of either L-leucine U-^{14}C (specific activity 7.95 μc/μmole) or L-phenylalanine-1-^{14}C (specific activity 0.42 μc/μmole) was added. The suspensions were incubated on the shaker for one hour and the cells then killed by boiling for 10 min. This cell suspension was centrifuged, washed and the resultant pellet fractionated with cold 5% TCA, ethanol/ether 3:1 (v/v), hot 10% TCA, and 0.2 M NaOH as described previously (GOTTLIEB and VAN ETTEN, 1966). All of the fractions were neutralized where necessary and the radioactivity of aliquots determined in Bruno's scintillation solution (BRUNO and CHRISTIAN, 1961) with a Packard Tri-Carb Spectrometer. Cab-o-sil (Packard Instrument Co., Inc., LaGrange, Ill.) was added when the protein fraction was counted. The results are expressed as μμmoles amino acid/mg protein/hr. All experiments contained at least three replicates.

Results

Phenylalanine and leucine were used for the incorporation studies, since they are not readily metabolized into compounds other than protein. As would be expected most of the radioactivity was recovered in either the soluble or protein fraction (Table 1). The one exception was for the incorporation of leucine by *R. solani* where most of the label was found in the protein fraction.

Table 1. *Percentage of the radioactivity from incorporation of phenylalanine-1-^{14}C and leucine-U-^{14}C into the various cell fractions of* R. solani *and* S. bataticola

Organism	Amino Acid	Fraction			
		Percentage of radioactivity[a]			
		Cold TCA	Ethanol/ether	Hot TCA	0.2 M NaOH
R. solani	phenylalanine	48	7	4	42
	leucine	7	4	3	87
S. bataticola	phenylalanine	38	13	5	43
	leucine	30	11	5	54

[a] Average value for all age groups.

The specific activities of both soluble and protein amino acids decreased with age in all cases (Table 2) with the greatest decrease usually occuring between the youngest and next to youngest cells. Phenylalanine was incorporated into the protein fraction more readily than leucine in both fungi.

The ratio of specific activity of the soluble amino acids to the protein amino acids increased with increasing age in *R. solani* indicating that the amount of amino acids incorporated from the internal amino acid

Table 2. *Specific activities of the soluble fraction and protein fraction of cells of different ages of* R. solani *and* S. bataticola *after incubation with* ^{14}C *amino acids*

Organism	Age, hrs.	μμ moles amino acid incorporated/mg protein/hr					
		Soluble fraction		Protein fraction		Ratio soluble/protein	
		phe	leu	phe	leu	phe	leu
R. solani	0— 16	930	26	1664	367	0.56	0.071
	16— 31	564	19	705	335	0.80	0.057
	31— 43	478	16	255	248	1.88	0.065
	43— 56	331	16	166	167	2.00	0.096
	56— 80	339	17	123	101	2.75	0.170
S. bataticola	0— 28	790	141	701	303	1.13	0.47
	28— 57	396	93	590	140	0.67	0.67
	57— 78	380	74	433	112	0.88	0.66
	78—102	453	68	481	114	0.94	0.60
	102—144	338	79	463	141	0.73	0.56

pool into protein decreased with age. However, in the case of *S. bataticola* there was very little change in this ratio with age.

Discussion

The results support the concept that after entering the cell these amino acids enter an internal pool from which they are removed for protein synthesis without being readily metabolized and incorporated into other fractions. In all experiments, the specific activities of the soluble fraction decreased with age. This change in the size of the internal amino acid pool with age agrees with data of other investigators who have studied the fungi (BENT and MORTON, 1964; DAWSON, 1965; GOTTLIEB and VAN ETTEN, 1964, 1966; KING and ISAAC, 1964; MEYERS and KNIGHT, 1961; PILLAI and SRINIVASAN, 1956). Such decreases might have resulted from reduction in uptake or more rapid incorporation of the amino acids into protein.

The reduction in specific activity of the protein fraction with increasing age of the cells indicates that there might be a change in the functioning protein synthesizing system. Such results are consistent with other data on fungi (BENT and MORTON, 1964; GOTTLIEB and VAN ETTEN, 1964, 1966; SUSKIND and BONNER, 1960). As would be expected the percentage decrease varied with the fungal species and the amino acid being incorporated. Whether decreases similar to those with L-phenylalanine and L-leucine occur with other amino acids is not known.

Our experiments indicate that the greatest decrease in the specific activities of the two fractions usually occurred between the youngest and the next to youngest cells. It is interesting that a similar decrease in the ergosterol content was found in both fungi. It has been suggested

that ergosterol may be associated with the cell membrane and hence be able to alter the cell permeability properties (GOTTLIEB and VAN ETTEN, 1966). Oxygen consumption in *R. solani* also had its greatest decline in the same period (VAN ETTEN *et al.*, 1966), a fact which might be important in the generation of energy, necessary for anabolic activities.

The ratio of the specific activity of the soluble fraction to that in the protein fraction increased with age in *R. solani* with both amino acids, in other words, the amount of amino acids incorporated from the internal pool into protein decreased quicker than the pool size. One therefore concludes that the limitation of protein synthesis is caused more by the protein synthesizing system itself than by the supply of amino acids available from the pool.

The ratio, however, remained fairly constant in the experiments with *S. bataticola*. Here the relative amount of amino acids incorporated from the pool into protein remained fairly constant for all age groups. Therefore, it seems as if the protein synthesizing activity might be less affected by aging than by the uptake of amino acids. This concept agrees with the observation by OBRIG (1967), that there is a decrease in permeability to glucose with increasing cell age in *S. bataticola*.

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