Cellular Basis of Growth Rate Differences in Isolates of Rhizoctonia solani: Metabolic Processes and Growth Rates

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ABSTRACT

The pathogen, *Rhizoctonia solani,* includes some strains which differ in their relative growth rates, independent of their ability to utilize nutrients and plant hormones or to respond to temp changes. The vigor of these isolates is correlated with the rate of synthesis of their lipids, proteins, nucleic acids, and cell wall material. The rate of synthesis of these metabolic reactions is in turn related to the uptake of the substrates from the medium, and seems to be a function of the cell membrane. When, for example, whole cells were used, there was a positive correlation between the relative growth of slow-, medium-, and fast-growing isolates with the synthesis of protein, but no such correlation was found when the relative rates of protein synthesis in cell-free preparations were measured.

The differences in permeability do not seem to be dependent on the amount of energy that is available to the different isolates of *R. solani,* for previous studies have shown the lack of correlation between the ATP content of the mycelium and relative growth rate. Furthermore, the current evidence indicates that even with the nonmetabolizable sugar analogues, 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose, the three isolates produce only insignificant amounts of carbon dioxide. Nevertheless, the uptake of these sugars by the isolates is correlated with their relative growth rates.

Other interesting positive relationships with growth are in the relative proportions of membrane-bound ribosomes, the amount of newly synthesized RNA, the rate of synthesis of the different types of RNA, and the content of CMP and UMP in the RNA. The ratio of 25S to 18S RNA is 1.4, a value much lower than has been reported for other species. The nucleotide composition of the *R. solani* isolates was similar to that of a number of other fungi with a G+C molar percentage of about 50. Other nucleotide data either are not correlated with growth rate of the isolates, or have a negative correlation. Uridine could be derived from exogenous uracil, and the rate of conversion to uridine is in the same order as the relative growth rates of the different isolates.

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A study of factors that determine differences in growth rates of a number of strains of *Rhizoctonia solani* Kühn has indicated that some of them are nutrient-dependent, but that others have relative growth rates that are independent of nutritive requirements. The limitation on slow-growing isolates was not caused by a need for macro or micronutrients, exogenous plant growth hormones, or other growth-promoting metabolites. The growth rate differences also were not determined by the different growth temp optima. The low relative growth rates could not be attributed to the cellular concn of protein, DNA, and RNA, or to the energy that had been stored as ATP.

Among the few studies that have been made on the cause of differences in growth rate are those with mutants that have both a slow growth rate and an impaired cellular metabolism. If mutants are accepted as artificial equivalents of natural isolates of a fungus, these mutants furnish a precedent for the concept that slow growth rates are dependent on a low rate of reaction in various physiological or biochemical systems. There is, for example, the case of the *petite* mutants of *Saccharomyces cerevisiae*. These yeasts grow very slowly, probably because of an impaired cytochrome and a weak succinic oxidase system (8, 9). Similarly, the *poky* mutant of *Neurospora crassa* is very slow-growing, and has a deficient terminal electron transport system. This microbe accumulates large quantities of cytochrome c but lacks cytochromes a and b. In addition, it has low succinic

acid and cytochrome c oxidase systems (16). The mycelium is low in protein, nucleic acids, and free glucose compared to the normally growing wild strain (23).

This paper reports the results of our studies on various biochemical activities of three isolates of *R. solani* in which relative growth is independent of nutritive factors.

MATERIALS AND METHODS.—In our previous paper are described the sources of our fast-, medium-, and slow-growing isolates, as well as the general procedures used in their culture (21). Except where otherwise indicated, the medium used in the present study was the glucose-casamino acid-vitamin medium (GCV). For studies on the oxidation of glucose and the tracing of synthetic products, 1.0 (wet wt) of mycelium was cut from the isolate growing in liquid medium and transferred immediately to a flask containing 50 ml of GCV medium in which cold glucose had been replaced with undiluted D-glucose-U-^{14}C (5 μCi). The production of carbon dioxide under aerobic conditions was a measure of the oxidation of the sugar, and the gas was trapped in 0.5 ml hyamine hydroxide (11).

The cells were fractionated by methods similar to those described by Gottlieb and Van Etten (12). RNA was estimated by the orcinol test (2), DNA by the diphenylamine test (30), and protein by the method of Lowry et al. (22), using yeast RNA, calf thymus DNA, and bovine albumin as standards, respectively. To study the composition of RNA, extracts were prepared by the method of Greenman et al. (13). Unless otherwise stated, the ages of colonies of the fast-, medium-, and slow-growing isolates were 3, 4, and 8 days, respectively. Harvested cells were immediately frozen in liquid nitrogen and ground into a fine powder.

The nucleotides from the total RNA hydrolysis were separated on precoated polyethylenimine-impregnated cellulose MN 300 plastic sheets (Brinkmann Instruments, Inc.), developed with 0.1 N formic acid, followed by 5.0 N formic acid (13). The nucleotide spots were eluted with 1.7 M $MgCl_2$ - 2.0 M Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4 (100:1, v/v), for 30 min at room temp (28). The eluted samples were then forced through a syringe filter containing Whatman No. 40 filter paper and read at the wavelength of maximum absorption. The extinction coefficients of the various nucleotides in this solution are: 2'- + 3'-CMP (λ_{max}, 270 nm) = 8.3×10^6, 2'-AMP (λ_{max}, 261 nm) = 15.3×10^6, 3'-AMP (λ_{max}, 261 nm) = 16.3×10^6, 2'- +3'-GMP (λ_{max}, 254 nm) = 13.1×10^6, and 2'- + 3'-UMP (λ_{max}, 262 nm) = 8.5×10^6. Portions of the eluted samples were counted for radioactivity.

The bases and nucleosides were separated by two-dimensional chromatography on Eastman cellulose chromatographic sheets with fluorescent indicator (28). The solvents, in order of use in developing the chromatograms, were *n*-butanol-water (86:14, v/v) and methanol-HCl (sp gr 1.18)-water (70:20:10, v/v) (28).

Total RNA was extracted from the fast isolate after 3 days of growth, from the medium isolate after 4 days, and the slow one after 8 days. For pulse labeling, a composite 2.5 g of mycelial mats from replicated flasks of GCV medium were incubated in 125 ml GCV medium containing uracil-2-^{14}C (1.2 μCi/ml) for 3 h at 25.5 C. For the chase experiment, pulse-labeled mycelia were partially dried, washed with 100 ml of GCV medium, and immediately transferred to 1.0 liter of GCV medium containing 3 mM nonradioactive uracil and incubated for 1.5 h. The cells were collected on Whatman No. 1 filter paper in a Buchner funnel and washed with distilled water at 4 C.

These cells, either fresh or frozen, were extracted as described by Eaton (7). All equipment which came into contact with the cells or extracts were autoclaved to minimize the activity of possible contaminating ribonucleases. Phenol was used to isolate RNA (20). The concn of RNA was determined spectrophotometrically by measuring the E_{260}/E_{280} at suitable dilutions (1).

The species of RNA were separated by electrophoresis on 2.4% preswollen polyacrylamide gels (4). An RNA solution containing about 30 μg was applied to gels and a current of 5 ma/gel (Canalco Model 300B Constant Current Power Source) was applied for 105 min at room temp.

After electrophoresis, gels were transferred to quartz cuvettes and scanned by ultraviolet (UV) light at 260 nm using a Gilford Linear Transport attachment coupled to a Beckman DU spectrophotometer. The gels were next transferred to stainless steel troughs with the same dimensions as the cuvettes. After removal of excess water, the gels were frozen by placing the troughs on powdered dry ice. Frozen gels were transferred to the platform of a gel-slicer (The Mickle Laboratory Engineering Co., Goomshall, Surrey, U.K.) and cut into 0.7-mm slices. These sections were placed on 2.4-cm filter papers and allowed to dry at 70 C in scintillation vials. Ten ml of scintillation solution consisting of 3 g BBOT (2,5-bis-[2-(5-tert-butylbenzoxazolyl)]-thiophene) in 1 liter toluene were added to each vial. The counting data presented were not corrected for background. The relative amounts and approximate specific radioactivities of different species of RNA were determined by cutting out the appropriate absorption and radioactivity peaks and weighing them.

Cell-free extracts for protein synthesis were prepared from sections of washed 4-day-old mycelial mats of *R. solani,* and then treated as described previously (27). The supernatant fluid from the 35,000 $\times$ *g* centrifugation (S-35) was used immediately for the study of both the in vitro protein synthesis, and the sucrose density-gradient sedimentation profiles of ribosomes. The concns of protein and nucleic acids in the S-35 fraction were estimated by determining the absorption at 260 and 280 nm at a suitable dilution, as described above (1).

All the glassware used in the preparation of S-35 fraction was washed in double-distilled water and sterilized, and the buffer was sterilized by Millipore filtration.

The Sephadex column procedure (6) was modified (10) and adapted for the cell-free synthesis of protein. A column made from the barrel of a 2.5-ml glass syringe (1 cm in diam) was prepared with a 4-cm-high bed of Sephadex G-25 superfine (Pharmacia Fine Chemicals, Inc.). The column was equilibrated with buffer 1 by passing 1 ml through five times; then 2.0 ml of an incubation mixture of L-phenylalanine-U-^{14}C (0.5 μCi), ATP (1.5 μmoles), and GTP (0.1 μmole) in buffer 1 was placed over the gel bed and allowed to run down the

column. A mixture of S-35 cell fraction (0.1 ml) and poly U (60 μg in 25 μl buffer) was then placed on the column. The column was eluted with buffer 1 and the protein eluate collected in a 15-ml test tube which contained 2 ml of a solution of 1% (w/v) unlabeled L-phenylalanine in 16% TCA. The final volume of the eluate-TCA solution was about 6 ml. When cycloheximide or puromycin was added, it was incorporated into the incubation mixture, whereas added ribonuclease (120 μg) was mixed with the S-35 fraction just before loading the column. All the compounds were used as solutions in buffer 1 and allowed to reach 25.5 C.

The zero time control was obtained by pipetting a mixture of S-35 and poly U directly into the tube containing TCA solution. After this, the elution of the column was started. The eluate in each tube was heated for 10 min at 90-95 C, cooled in an ice bath, and the precipitate collected on a Millipore filter, type HA (0.45-μ pore diam). The precipitates were washed with five 5-ml portions of cold 5% TCA. The filters were placed in scintillation vials, dried for 2 h at room temp, then at 100 C for 10 min, and finally cooled to room temp. Ten ml of BBOT-toluene fluid was added to each vial and the radioactivity was measured as described above.

The incubation mixture for studies on the incorporation of L-amino acid-U-^{14}C mixture by endogenous and natural messenger differed from the above described mixture in that the labeled phenylalanine was replaced with 0.5 μCi of an L-amino acid-^{14}C mixture. This amino acid mixture was supplemented with 0.005 μmole of each of six other unlabeled amino acids (L-tryptophan, L-asparagine, L-glutamine, hydroxy L-proline, L-methionine, and L-cysteine) in which it was deficient. The eluted protein was collected in TCA containing 1% (w/v) casamino acids. The procedures for the isolation and counting of radioactive protein were the same as described for the poly U system.

Ribosomes were obtained from the S-35 cell-free extract by sucrose density-gradient sedimentation. Membrane-bound ribosomes were extracted by adding buffer 1 containing 0.5% sodium deoxycholate to the pellet obtained after centrifugation at 17,000 × *g*. Aliquots of S-35 fraction containing about 16 OD_{260} units were layered over linear sucrose gradients (10-30% sucrose in buffer 1) with 1.0 ml 35% sucrose solution as cushion in cellulose nitrate centrifuge tubes. The tubes were centrifuged at 185,000 × *g* in an SW-41 rotor of a Spinco Model L-2 ultracentrifuge for 90 min at 4 C. The distribution of ribosomes in the sucrose gradients was determined at 254 nm and a flow rate of 0.6 ml per min with an ISCO Model 180 density-gradient fractionator and a recording spectrophotometer (Model UA-2) with a 1-cm light path.

The uptake of metabolites by whole cells of *R. solani* was determined in the same experiments as their synthesis into macromolecules and degradation into carbon dioxide. One gram (wet wt) portions of mycelium were added to 250-ml Erlenmeyer flasks containing 50 ml of sterile GCV liquid medium. To each flask, in triplicate series, was added one of the following: L-phenylalanine-U-^{14}C, 5 μCi; L-leucine-U-^{14}C, 5 μCi; orotic acid-6-^{14}C, 1 μCi; or thymidine-methyl-^{3}H, 10 μCi. The incubation times for these labeling studies were 1 h for the amino acids and, unless otherwise stated, 2 h for the remaining compounds. The cells were then washed five times with ice-cold distilled water, dried in a vacuum oven at 80 C to constant wt, and fractionated as previously described. A portion of each fraction was measured for radioactivity. The scintillation solution was composed of 600 ml toluene, 385 ml 95% ethanol, 3.44 gm 2,5-diphenyloxazole (PPO) and 14.8 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP). All counting data are presented as corrected for background. The uptake of precursors is given only by measurements of the amount of radioactivity in the cells since in most cases measurements of disappearance from the medium was too small for accurate determination.

When D-glucose-U-^{14}C was used, it replaced the unlabeled sugar in the GCV liquid medium. Nonmetabolizable glucose analogues were used in a similar fashion as described above. One gram (wet wt) of mycelium from a 4-day-old culture was first added to the 50 ml of medium. After 10-min equilibration, 3-*O*-methyl-^{14}C-D-glucose (3-*O*-MG) or 2-deoxy-D-glucose-1-^{14}C (2DOX) was added to make a final concn of 5 μM or 0.75 μM, respectively. Evolved carbon dioxide was absorbed by hyamine hydroxide, and the labeling proceeded for the various periods indicated in the section on results. The mycelium was then treated and radioactivity measured as described above.

Source of reagents.—Many of the special reagents were purchased from either Sigma Chemical Co. or Mann Research Laboratories. The hormones were obtained from Calbiochem, puromycin from Nutritional Biochemical Corp., and actidione was a gift from the Upjohn Co. Polyacrylamide chemicals were purchased from BioRad Laboratories. The radiochemicals were from New England Nuclear Corp., Schwarz Bioresearch,

TABLE 1. Uptake and incorporation of D-glucose-U-^{14}C into various cell fractions by 4-day-old colonies of three *Rhizoctonia solani* isolates having different growth rates

Isolates (Growth rate types)	$^{14}CO_2$(cpm) evolved/ g (dry wt) cells	cpm/mg dry wt cells						
		Whole cells	80% Ethanol	Ether: Ethanol	Cold TCA	Hot TCA	0.2 N NaOH	Residue
Fast	88,380(10.1)[a]	5,170(7.0)	2,840(8.2)	124(4.1)	615(5.0)	870(5.1)	655(6.7)	102(4.9)
Medium	52,760 (6.0)	1,880(2.6)	780(2.3)	33(1.1)	248(2.0)	350(2.1)	330(3.4)	32(1.5)
Slow	8,770 (1.0)	735(1.0)	345(1.0)	30(1.0)	124(1.0)	170(1.0)	98(1.0)	21(1.0)

[a]Figures in parentheses represent the relative ratios in each column.

Inc., and Amersham/Searle Corp.

RESULTS.—*Incorporation and metabolism of labeled glucose.*—The relative growth rates of the three isolates of *R. solani* were strongly related to the ability of these organisms to oxidize glucose to carbon dioxide. In the same experiment, the fast-, medium-, and slow-growing isolates weighed 317, 131, and 21 mg, respectively, and the ratio of their mass was 15.1:6.2:1.0. The carbon dioxide which they produced decreased in the same order, with a ratio of 10.1:6.0:1.0 (Table 1). Similar results were obtained with colonies of different ages. For the fast-, medium-, and slow-growing cultures from which mycelia were taken after 3, 4, and 8 days, the ratio of carbon dioxide evolved was 9.3:6.2:1.0. When peripheral hyphae from 4-day-old mats of the three isolates were used, the differences in carbon dioxide production were not as great, but the ratio was still in the same direction as growth, 2.8:2.4:1.0.

The 80% ethanol extract of the 4-day-old mycelia that had been exposed to radioactive glucose contained ^{14}C with cpm in the same order as the growth of the different isolates, 8.2:2.3:1.0 (Table 1). About 50% of the radioactive material that was in the mycelia was recovered in this extract. Probably a good deal of the radioactivity was from the sugar that still remained unconverted in the soluble pool. The ratio in the TCA extracts was 5.0:2.0:1.0; these extracts should have contained any remaining sugar, the synthesized amino acids, nucleosides, nucleotides, purine and pyrimidine bases, and other small acid-soluble molecules, as well as polysaccharides.

Comparatively small amounts of lipids were synthesized during the incubation period, as shown by the low cpm in the ethanol-ether fraction compared to fractions containing nucleic acids, proteins or small molecules. Despite this, the amount of synthesized lipids decreased with decreasing growth ability of the isolates, and gave a ratio (for example) of 5.3:2.9:1.0 (Table 1).

The greatest synthetic activity was generally found in the materials present in the hot TCA-soluble fraction, the nucleic acids. The ratio in order of decreasing growth rate of the isolates was 5.1:2.1:1.0 (Table 1). In other experiments carried out in other ways, the actual ratios were different, but were always in the same direction.

Similar results occurred with the 0.2 N NaOH extract which represented the synthesis of protein; for the fast-, medium-, and slow-growing isolates a typical ratio was 6.7:3.4:1.0 (Table 1). Labeling in the residues from these extractions is held to represent cell wall material and other insoluble compounds. The cpm in this material also was in accord with the relative growth rates, decreasing with the decreasing growth rates of the isolates (Table 1).

TABLE 2. Uptake and incorporation of L-phenylalanine-U-^{14}C into various cell fractions by 4-day-old colonies of three *Rhizoctonia solani* isolates having different growth rates.

Isolates (Growth rate types)	cpm/mg (dry wt) cells		
	Whole cells	80% Ethanol	0.2 N NaOH
Fast	1,550(6.5)[a]	154(1.6)	1,070(7.5)
Medium	740(3.1)	97(1.0)	600(4.2)
Slow	240(1.0)	95(1.0)	143(1.0)

[a]The figures in parentheses represent the relative ratios in each column.

Synthesis of protein.—The greater synthesis of protein with the higher growth rates of the different isolates of *R. solani* occurred not only in its de novo synthesis from glucose, but also in its formation from the preformed amino acids, L-phenylalanine and L-leucine. The respective ratios for their incorporation were 7.5:4.2:1.0 and 3.1:2.3:1.0 (Table 2).

In contrast, the results from cell-free protein synthesis did not correlate with the relative growth rates of the fungi. The fastest-growing isolate had the lowest synthesis and the slowest-growing isolate had the highest rate, under conditions when the natural message was used and the reaction was carried out on a Sephadex column (Table 3). With polyuridylic as the artificial message, the fast isolate had the greatest incorporation, the medium isolate had the lowest, and the slowest had an intermediate value (Table 3). The results remained in this order whether incorporation was calculated on the basis of protein or nucleic acid (Table 3). That the cell-free protein synthesis was real is indicated by its dependence on the presence of ATP and GTP, as well as its susceptibility to ribonuclease. The synthesis of peptides was not inhibited in either system by cycloheximide. When natural message was used, the reaction was only slightly inhibited by puromycin, but was inhibited from

TABLE 3. Incorporation of L-amino acid-U-^{14}C mixture into protein and nucleic acid by cell-free systems from three *Rhizoctonia solani* isolates having different growth rates[a]

Experimental conditions		Amino acid-U-^{14}C mixture incorporation (cpm/mg protein)			Amino acid-U-^{14}C mixture incorporation (cpm/mg nucleic acid)		
		Fast	Medium	Slow	Fast	Medium	Slow
Endogenous message	After elution	1,310	1,630	3,760	3,930	5,140	12,110
	0-time control	66	69	80	199	218	300
Poly U message	After elution	2,930	430	980	8,780	1,350	3,660
	0-time control	63	44	67	190	139	254

[a]In these experiments, 0.1 ml S-35 cell fraction contained: for fast-growing isolate, protein 9.995 mg, RNA 0.332 mg; for medium-growing isolate, protein 1.195 mg, RNA 0.380 mg; for slow-growing isolate, protein 0.810 mg, RNA 0.216 mg.

51 to 66% in the poly U-directed synthesis.

Synthesis of nucleic acids.—Further examination of cellular RNA synthesis using uracil-2-^{14}C as the precursor, showed that on a dry wt basis its incorporation followed the rates of growth of the three isolates. This relationship held true in both the acid-soluble extract and the alkaline hydrolysate (Table 4). The amount of uracil in the RNA showed no consistent trend with growth rates. But the uridine content on a mg dry wt basis did relate to the order of the growth rates of the isolates, and these values, as cpm, were fast, 2,014; medium, 1,014; and slow, 552. Cytosine and cytidine had labeling too low to be meaningful.

The mole percentages of UMP and GMP in the hydrolysates of the total RNA of the cells increased with increasing growth rates, but did not vary in any consistent way with the differences in growth rates of the isolates (Table 5). The molar percent of the sum of GMP and CMP was always more than 50. The ratios for (A + U)/(G + C), (A + G)/(U + C), A/U, and G/C did not correlate with the rates of growth of the isolates. Using labeled uracil as the precursor, the conversion to uridine monophosphate and cytidine monophosphate decreased with decreasing relative growth rates, the respective values in cpm being 30,356, 9,508, 2,695 and 19,401, 5,841, 1,253 for fast, medium, and slow isolates.

Labeling the pyrimidine components of the nucleotides and nucleic acids with orotic acid-6-^{14}C gave results similar to the labeling with glucose or uracil except that the fast and medium isolates of the fungus had proportionately more label (Table 6). Attempts to label the DNA and nucleotides with thymidine were unsuccessful. The total uptake of the precursor by the fast isolate was only 273 cpm per mg cell dry wt, and there were no obvious differences in the labeling among the three isolates. The radioactivities in the cold TCA and the hot TCA fractions and in all isolates were only between 23 and 78 cpm.

Polyacrylamide gel electrophoretic profiles showed that *R. solani* contained all the species of RNA usually found in eucaryotic organisms. These were ribosomal heavy (25S) and light (18S), mitochondrial heavy (23S) and light (16S), and a mixture of 4S and 5S which were not separated. In addition, some components had small peaks slightly above 25S and also some peaks that might indicate polydispersed RNA between the 5S and 16S peaks. The species of RNA and their relative proportions were similar whether derived from pulse or chase experiments or determined by optical density and radioactivity measurements. Typical curves are shown in Fig. 1. Comparison of results from the three isolates, showed no regular differences that could be correlated with the growth rates of the different isolates when the optical densities of the different isolates were compared. However, measurements of total radioactivity revealed that higher rates of synthesis were related to higher growth rates; for example, for uracil-2-^{14}C the cpm/mg protein were 1,800, 629 and 338 for fast, medium, and slow isolates, respectively. The ratio of 25S to 28S was always about 1.4:1.0 which is lower than has been reported for other organisms.

Attempts to determine whether the growth of the isolates and their cellular synthesis of protein were related to differences in the sizes of the membrane-associated ribosomes, resulted in no rational pattern. The extracts from fast- and slow-growing isolates had monosomes and polysomes of various sizes in both membrane-free and membrane-bound material except for the extract from the bound material of the slow-growing isolate. The latter had a profile which contained only a ribosomal peak and no polysomes or subunits (Fig. 2). In contrast, the medium-growing isolate showed only a trace of the subunits and no polysomal peaks.

Permeability.—The uptake of glucose from the medium into the cells followed the same trend as the growth rates of the different isolates (Table 1). The 80% ethanol extracts probably also were indicative of uptake and reflect unaltered glucose. A similar trend of uptake by the different isolates is shown with orotic acid (Table 6), but no differences were found with thymidine. Using the amino acids as precursors for protein synthesis by whole cells, the 80% ethanol extracts did not show a decrease in cpm with decreasing growth rate of the fungal isolates. With phenylalanine in two different types of studies, there was no difference between the medium- and slow-growing isolates but the fast-growing isolate always had greater activity. With leucine as precursor, there was very little

TABLE 4. Radioactivity of the acid-soluble extract and the alkaline hydrolysate of RNA from uracil-2-^{14}C pulse-labeled (3 h) mycelium of three *Rhizoctonia solani* isolates having different growth rates

Isolates (Growth rate types)	cpm/mg (dry wt) of mycelium	
	Acid-soluble extract	Alkaline hydrolysate
Fast	69,308(3.9)[a]	44,954(14.0)
Medium	30,897(1.7)	13,830 (4.3)
Slow	17,778(1.0)	3,211 (1.0)

[a]The figures in parentheses represent the relative ratios in each column.

TABLE 5. Nucleotide composition of the total RNA from three *Rhizoctonia solani* isolates having different growth rates

Isolates	Nucleotide proportions[a]							
	moles %							
	A	G	C	U	A+U/G+C	A/U	G/C	A+G/U+C
Fast	18.9	27.5	26.4	27.2	0.86	0.70	1.04	0.87
Medium	20.1	27.6	25.6	26.7	0.88	0.75	1.08	0.91
Slow	19.9	32.1	24.0	24.0	0.78	0.83	1.34	1.08

[a]A = 2′+3′-AMP; G = 2′+3′-GMP; C = 2′+3′-CMP; U = 2′+3′-UMP.

difference between the fast- and medium-growing isolates.

Permeability studies conducted with metabolizable precursors are difficult to interpret, because permeability is dependent, not only on the ability of the substrate to permeate the cell, but also on the diminishing internal concn of the substance as it is being catabolized or in other ways transformed. Experiments were therefore done with the nonmetabolizable sugars, 3-*O*-methyl-^{14}C-D-glucose and 2-deoxy-D-glucose-1-^{14}C. The uptake of both glucose analogues by *R. solani* was linear for at least 90 min. A 30-min labeling period was used in all subsequent studies of permeability. The data show that the rate of uptake of the two sugar analogues paralleled the order of growth rate of the three fungal isolates as measured by the cpm content of the cells (Table 7). When glucose was not included in the medium and 3-*O*-MG was the only labeled material, the ratio in order of decreasing growth was 27.0:4.3:1.0, and with 2DOX alone, 14.7:9.2:1.0.

When glucose was also included in the medium, the relative uptake was in the same direction as the growth rates of the three isolates, with ratios for the fast-, medium-, and slow-growing isolates for 3-*O*-MG and 2DOX of 19.9:3.9:1.0, and 14.3:7.2:1.0, respectively.

With both sugar analogues, negligible radioactivity was detected in the expired carbon dioxide, about 20 cpm per 100 mg dry wt of mycelium over the 30-min labeling period. Thus, one can conclude that in *Rhizoctonia,* too, these sugar analogues were not significantly metabolized.

TABLE 6. Uptake and incorporation of orotic acid-6-^{14}C into various cell fractions by 4-day-old colonies of three *Rhizoctonia solani* having different growth rates.

Isolates (Growth rate types)	cpm/mg dry wt cells		
	Whole cells	Cold TCA	Hot TCA
Fast	2,580(33.1)[a]	1,210(21.6)	900(52.9)
Medium	515 (6.6)	280 (5.0)	140 (8.2)
Slow	78 (1.0)	56 (1.0)	17 (1.0)

[a]The figures in parentheses represent the relative ratios in each column.

DISCUSSION.—Since our previous studies have shown that the relative growth rates of different isolates of *R. solani* cannot always be explained by deficiencies in their ability to utilize different nutrients, by the need of some isolates for exogenous vitamins and hormones, or by their specific reactions to other environmental conditions, it is not surprising to find that such growth differences are correlated with the metabolic activities of the organisms (21). In the current experiments, this correlation is seen both in the catabolism of glucose to carbon dioxide and in the utilization of that substrate for the synthesis of lipids, nucleic acids, protein, cell wall residue, and other metabolites. Confirmation of this relationship among the growth rates of the three isolates is also seen in the more direct incorporation of precursors

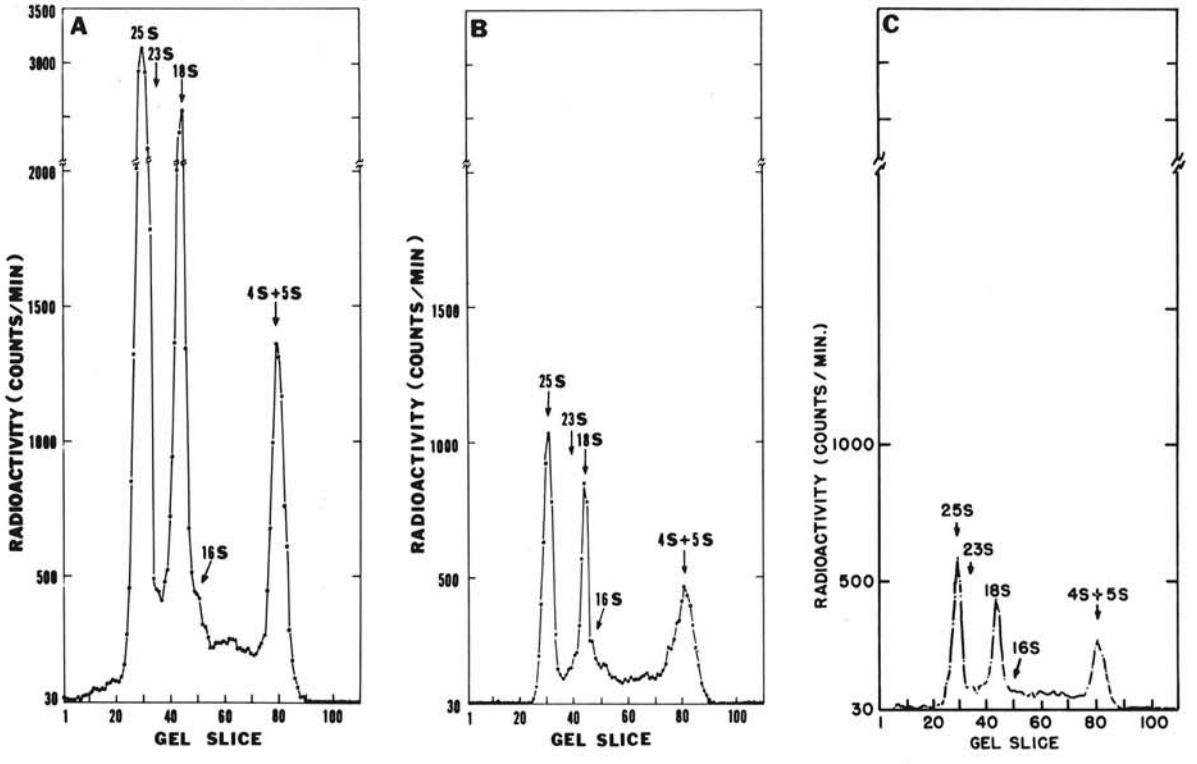

Fig. 1-(A to C). Radioactivity profiles from polyacrylamide gels of RNA extracted from pulse-labeled cells of three *Rhizoctonia solani* isolates having different growth rates: **A)** fast-; **B)** medium-; and **C)** slow-growing.

(such as amino acids) into protein, and uracil or orotic acid into RNA. Yet, even the correlation of both catabolism and the different syntheses with growth rates cannot be accepted per se as proof of their primary involvement in bringing about the differences in growth. Protein synthesis is a good example of this difficulty, for even though the cellular synthesis of these macromolecules is correlated with greater growth rates, this relationship did not occur when cell-free extracts were used with either a natural or an artificial message.

TABLE 7. Uptake of 2-deoxy-D-glucose-1-^{14}C and 3-*O*-methyl-^{14}C-D-glucose in glucose-free medium by 4-day-old colonies of three *Rhizoctonia solani* isolates having different growth rates

Isolates (Growth rate types)	pmoles taken up/mg dry wt cells/30 min	
	2-deoxy-D-glucose-1-^{14}C	3-*O*-methyl-^{14}C-D-glucose
Fast	19.26(14.7)[a]	88.02(27.0)
Medium	12.05 (9.2)	14.02 (4.3)
Slow	1.31 (1.0)	3.26 (1.0)

[a]Figures in parentheses represent the relative ratios in each column.

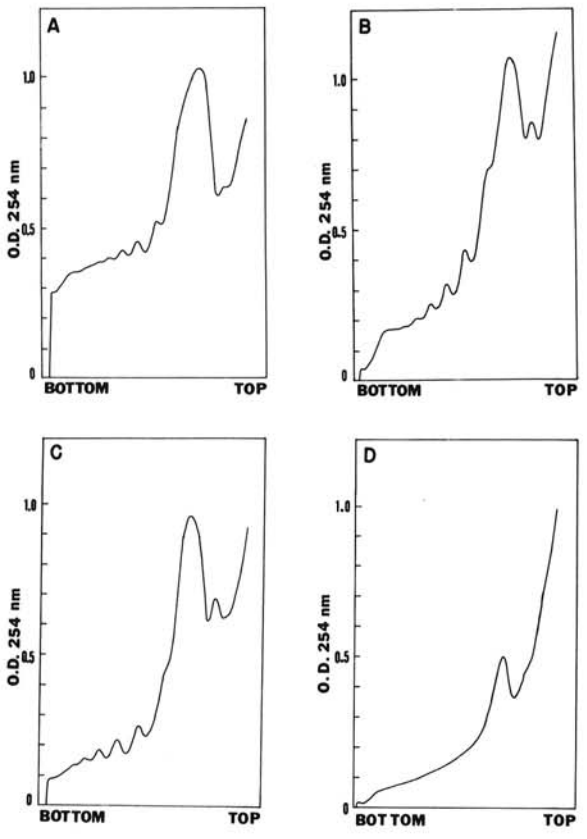

Fig. 2-(A to D). Sucrose density-gradient profiles of ribosome preparations from fast- and slow-growing isolates of *Rhizoctonia solani*. **A)** free ribosome preparation from fast isolate, **B)** membrane-bound preparation from fast isolate, **C)** free ribosome preparation from slow isolate, **D)** membrane-bound ribosome preparation from slow isolate. The prominent peak in each case corresponds to the 80S ribosomes, and the smaller peaks to the left are polyribosomes.

The hypothesis that the rates of protein synthesis by the isolates were dependent on the relative amounts of polysomes in the cells cannot be supported because in these experiments polysomes could not be detected in the medium-growing strain of the fungus, but were present in the rapid- and slow-growing ones.

The dependence of rapid protein synthesis on the presence of relatively high amounts of bound rather than free ribosomes has been shown for serum albumin (31) and for glycoproteins in the rat (15). This relationship may also hold for *R. solani,* since only the fast isolate contained membrane-bound polyribosomes.

The positive correlation between the synthesis of total RNA and growth rate resembles that found in bacteria (19, 25, 29). All the usual species of RNA were present in each isolate, and the amount of each positively correlated with growth rate. The ratio of 25S and 18S RNA was about 1.4:1.0 which is unusually low compared to *Ustilago maydis* (2.5:1.0), bean (2.4:1.0), and rat liver (2.8:1.0) (20, 26).

Nucleotide composition of the *R. solani* isolates was similar to that in other fungi, with a molar percentage for G + C of slightly more than 50 (17, 18, 24).

The data which show that many metabolic activities are higher in fast- than in slow-growing isolates of *R. solani* are interesting in themselves, but do not alone help separate the cause from effect in these phenomena. What is needed is a common function whose change might govern or limit the rate of activity of all the other metabolic processes. The differences in the permeability of *R. solani* to nutrients could be such a governing reaction, for in all experiments and with different precursors, the general positive relationship held between permeability and growth rate. The uptake of labeled glucose by the isolates, measured directly as radioactivity either in the cells, or in the 80% ethanol extracts, varied with growth rate, and similar results were found for amino acids and orotic acid as precursors for protein and nucleic acids, respectively. Furthermore, the correlation of growth rates with permeability also held for the nonmetabolizable analogues of glucose.

If the different rates of permeability were the common cause of the difference in growth rate of these three isolates, it would explain the results of other catabolic and anabolic reactions that are related to growth rate. Differences in permeability would then be the important factors in keeping the intercellular concns of nutrients at specific limiting levels, and would thus determine the amount of synthesis which would be possible from the nutrients.

Other evidence supporting the growth-limiting role of permeability is the data from cell-free protein synthesis. In these experiments, where there is no permeability barrier, there is also no correlation of the synthesis with growth rate, indicating the importance of the cell membrane in differential limitation of growth for the three isolates. Additional evidence supporting growth regulation by nutrient permeability is derived from the nonmetabolizable sugar experiments. In these experiments there is no degradation of substrate, and

therefore no synthesis of metabolites from the sugars and no production of ATP. Yet, with both compounds there was a direct correlation between the innate growth rate of the isolate and absorption of the sugar, measured in separate experiments.

Singling out any one factor as the cause of differences in growth rate is difficult because the cellular metabolic reactions are interdependent. A decrease in protein synthesis could bring about a decrease in permeability, since it might also lead to a decrease in the permease that is required for the transport of the substrate. Similarly, a decrease in energy storage compounds such as ATP might limit permeability because movement through the cell membranes is most often energy-dependent.

In slow-growing mutants of *Saccharomyces cerevisiae* and *Neurospora crassa* (the *petite* and *poky* strains, respectively) there are deficient mitochondrial mechanisms. Thus these mutants would have less energy available for growth (8, 9, 16, 23). The growth of a number of organisms has been shown to be proportional to the amount of ATP which was synthesized (3, 5, 14). However, in the *R. solani* isolates there was no correlation between the amount of ATP and growth, as measured on a dry wt basis, the basis for all measurements in this study. Instead, the converse occurred, a decrease of ATP correlated with increasing growth rates. The fact that the nonmetabolizable sugars, which cannot support ATP synthesis, are absorbed in positive correlation with growth rates strongly points to a governing role for permeability which is independent of the energy requirements.

The data from *R. solani* best support the hypothesis that differences in the growth rate of the three isolates of this fungus can be explained by their different permeability to nutrients. Those isolates that allow a high permeability have a strong metabolic ability, and thus vigorous growth. A limited permeability would then account for the relatively weak metabolic activities, and consequently slower growth rates of the other isolates.

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