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Effect of 3-Phenylpropanoic Acid on Growth of and Cellulose Utilization by Cellulolytic Ruminal Bacteria

ROBERT J. STACK* AND MICHAEL A. COTTA

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

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The growth of several cellulolytic species of ruminal bacteria was measured in media containing either cellobiose or cellulose as the energy source and with or without added 3-phenylpropanoic acid (PPA). With *Ruminoccoccus albus* 7 and 8, the addition of PPA greatly enhanced the rate of cellulose utilization but had little effect on the rate of growth when cellobiose was the energy source. Comparative rates of growth obtained on either cellobiose or cellulose for *Ruminococcus flavefaciens* FD1 or C94 and *Butyrivibrio fibrisolvens* 12, 49, or A38 were similar regardless of the PPA content of the growth medium.

Media used for the cultivation of ruminal bacteria are often supplemented with clarified rumen fluid as a nonspecific source of a variety of nutrients required by these organisms. Many of these nutrients have been identified, allowing the formulation of chemically defined media capable of supporting the growth of many ruminal bacteria (1, 5). Some examples of identified nutrients in rumen fluid include hemin for the growth of *Bacteroides ruminicola* (2), coenzyme M (mercaptoethanesulfonic acid) for the growth of *Methanobrevibacter ruminantium* (10), or various straight- and branched-chain volatile fatty acids for the growth of many species of ruminal bacteria (2).

Recently, Hungate and Stack (6) showed that 3-phenylpropanoic acid (PPA) dramatically stimulated the cellulolytic activity of *Ruminococcus albus* 8 when grown in a synthetic medium. The effect of PPA on *R. albus* 8 has been correlated with the ability of this organism to form high-molecular-weight enzyme complexes and a bacterial capsule (9).

Several workers have recently experienced difficulty in culturing *Ruminococcus* species on cellulose in a variety of defined media (7; D. Akin, personal communication). We therefore decided to examine the effect of PPA on the type strain of the species, *R. albus* 7 (3), as well as several other species of ruminal bacteria.

R. albus 7, *R. flavefaciens* FD1 and C94, *Butyrivibrio fibrisolvens* A38, 12, and 49, and *Bacteroides succinogenes* S85 were obtained from the culture collection of M. P. Bryant and R. B. Hespell, Department of Dairy Science, University of Illinois, Urbana. *R. albus* 8 was isolated and described in a previous report (6). Cultures were grown anaerobically at 37°C in the defined medium of Hungate and Stack (6) modified by the inclusion of the following volatile fatty acids (each at a 25 μM final concentration): *n*-butyric, *n*-valeric, isobutyric, isovaleric, and 2-methylbutyric. Trypticase (BBL Microbiology Systems, Cockeysville, Md.) at 2 g/liter was also included. Pebble-milled Whatman no. 1 filter paper or cellobiose (1.5 g/liter) served as the energy source, and PPA (Aldrich Chemical Co., Inc., Milwaukee, Wis.), when added to the medium, was present at a final concentration of 25 μM.

Cultures (6 ml) were grown in sealed tubes (16 by 125 mm) as described previously (6). Growth in cellobiose-containing medium was monitored by optical density measurements (600 nm). Cellulose utilization was monitored by the removal of small samples from cellulose-containing cultures at various times, with subsequent determination of residual carbohydrate by means of the anthrone procedure (4). Cellulose utilization was calculated as the difference between the cellulose concentration at zero time and the residual cellulose concentration at various times during growth. All cultures were inoculated with a 1% inoculum of a PPA-depleted stock culture grown on cellulose.

All of the ruminococci grew well in the defined medium of Hungate and Stack (6) when cellobiose was provided as the energy source. However, *Butyrivibrio fibrisolvens* grew poorly in this medium unless supplemented with Trypticase. Therefore, Trypticase was included in all experimental me-

TABLE 1. Effect of PPA on the growth of and rate of cellulose disappearance in pure cultures of ruminal bacteria

Organism	Specific growth rate constant (h^{-1})[a] when PPA was:		Rate of cellulose degradation (h^{-1})[b] when PPA was:	
	Absent	Present[c]	Absent	Present[c]
Ruminococcus alubus				
7	0.55	0.62	0.01	0.12
8	0.36	0.48	<0.01	0.11
Ruminococcus flavefaciens				
FD1	0.36	0.36	0.24	0.24
C94	0.17	0.17	0.03	0.03
Butyrivibrio fibrisolvens				
A38	0.43	0.43	ND[d]	ND
49	0.48	0.48	[e]	[e]
12	0.26	0.28	[f]	[f]

[a] Specific growth rate constant of cultures grown in cellobiose (1.5 g/liter)-containing medium. Values are means of duplicate experiments.
[b] Rate of cellulose degradation claculated from the slope of the natural logarithm of cellulose degraded versus time. Cultures were grown in cellulose (1.5 g/liter)-containing medium. Values are means of duplicate experiments.
[c] PPA present in the growth medium at 25 μM.
[d] ND, Not determined.
[e] Degraded 0.28 g of cellulose per liter in 20 days.
[f] Degraded 0.33 of cellulose per liter in 20 days.

* Corresponding author.

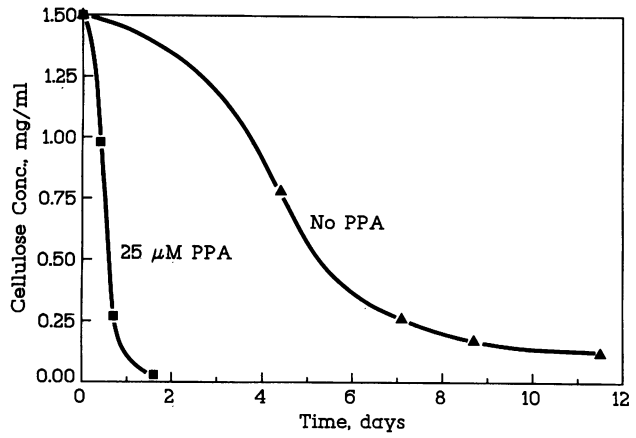

FIG. 1. Disappearance of cellulose over time in *R. albus* 7 cultures in the presence and absence of PPA. Cultures (6 ml) were inoculated with a 1% inoculum of a PPA-depleted stock culture of *R. albus* 7, and samples were withdrawn at selected times for cellulose analysis.

dia. Neither Trypticase nor yeast extract was found to contain detectable quantities of PPA, as determined by gas-liquid chromatography of methylated organic solvent extracts prepared from acidified 5% (wt/vol) aqueous solutions of these materials (6).

Considerable difficulty was encountered in obtaining reproducible growth of *Bacteroides succinogenes* S85 in cellulose-containing media. A long and variable lag period (2 to 9 days) preceeded a period of very rapid cellulose digestion (less than 24 h). This erratic growth occurred in both the presence and the absence of PPA or when 10% (vol/vol) rumen fluid was included in the media. Thus, an accurate interpretation of the effect of PPA on the growth of this organism was not possible.

With cellobiose-containing media, the growth rates of *R. albus* 7 and 8 were slightly stimulated by PPA, while the growth of none of the other organisms was so affected (Table 1). When cellulose was the energy source, however, PPA dramatically stimulated the rates of cellulose digestion by *R. albus* 7 and 8. PPA had little effect on cellulose digestion by the other species. The disappearance of cellulose over time in cultures of *R. albus* 7 with and without PPA in the medium is shown in Fig. 1. In the presence of PPA, approximately 90% of the cellulose was degraded in 24 h, and cellulose degradation was complete in less than 2 days, while cellulose degradation was incomplete even after 11 days when PPA was absent. These results are very similar to those previously reported for *R. albus* 8 (6).

R. albus 7 has been used widely over many years in rumen microbiological studies as a representative of one of the predominant species of cellulolytic ruminal bacteria. The present study shows that *R. albus* 7 requires PPA for rapid rates of cellulose digestion, as was shown previously for *R. albus* 8 (6). That this requirement has been overlooked until now probably reflects the fact that most workers routinely add rumen fluid or use cellobiose as the energy source in their media. In the former case, the high PPA content of rumen fluid (generally greater than 300 μM [6, 8]) coupled with the low levels required for optimal growth (3μM for *R. albus* 8 [6]) suggest that the PPA requirement for *R. albus* can be met even when rumen fluid is added at a level of but a few percent. When cellobiose is used, there is apparently no need for PPA, as the data in Table 1 show.

It now appears likely that most, if not all, *R. albus* strains exhibit greatly increased rates of growth with cellulose as an energy source when PPA is present. The results of Wood et al. (11) obtained from a study of the cellulases of *R. albus* SY3 are best explained on the basis of the PPA content of their media (9). Similarly, *R. albus* 69 (D. W. Fletcher, Ph.D. thesis, Washington State University, Pullman, 1956) required an unidentified factor contained in rumen fluid for growth on cellulose (but not cellobiose) in a defined medium. Since the cellulose digestion rates of *R. flavefaciens* are PPA independent (Table 1), PPA stimulation of cellulose digestion may provide a useful taxonomic distinction between *R. albus* and *R. flavefaciens*.

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LITERATURE CITED

1. **Bryant, M. P.** 1977. Microbiology of the rumen, p. 287-304. *In* M. J. Swenson (ed.), Duke's physiology of domestic animals, 9th ed. Cornell University Press, Ithaca, N. Y.
2. **Bryant, M. P. and I. M. Robinson.** 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. **84**:605–614.
3. **Bryant, M. P., N. Small, C. Bouma, and J. M. Robinson.** 1958. Characteristics of ruminal anaerobic cellulolytic cocci and *Cillobacterium cellulosolvens* n. sp. J. Bacteriol. **76**:529–537.
4. **Dische, Z.** 1962. Color reactions of carbohydrates, Methods Carbohydr. Chem. **1**:477–512.
5. **Hungate, R. E.** 1966. The rumen and its microbes. Academic Press, Inc., New York.
6. **Hungate, R. E., and R. J. Stack.** 1982. Phenylpropanoic acid: growth factor for *Ruminococcus albus*. Appl. Environ. Microbiol. **44**:79–83.
7. **Russell, J. B.** 1985. Fermentation of cellodextrins by cellulolytic and noncellulolytic rumen bacteria. Appl. Environ. Microbiol. **49**:572–576.
8. **Scott, T. W., P. F. V. Ward, and R. M. C. Dawson.** 1964. The formation and metabolism of phenyl-substituted fatty acids in the ruminant. Biochem. J. **70**:12-24.
9. **Stack, R. J., and R. E. Hungate.** 1984. Effect of 3-phenylpropanoic acid on capsule and cellulases of *Ruminococcus albus* 8. Appl. Environ. Microbiol. **48**:218–223.
10. **Taylor, C. D., B. C. McBride, R. S. Wolfe, and M. P. Bryant.** 1974. Coenzyme M essential for growth of a rumen strain of *Methanobacterium ruminantium*. J. Bacteriol. **120**:974–975.
11. **Wood, T. M., C. A. Wilson, and C. S. Stewart.** 1982. Preparation of the cellulase from the cellulolytic anaerobic bacterium *Ruminococcus albus* and its release from the bacterial cell wall. Biochem. J. **205**:129-137.