THE EFFECT OF OXALATE ON BACTERIA ISOLATED FROM THE RUMEN

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Summary

High oxalate levels showed no inhibitory effect on twenty-six apparently different strains of ruminal bacteria. One organism RO-16 decomposed oxalate. Examination of the filtrates and residues from the rumen content of sheep showed that the residues were more potent in oxalate decomposition than the filtrates. An organism resembling RO-16 was isolated from residues and filtrates which decomposed oxalate.

I. INTRODUCTION

It was suggested (Talapatra, Ray, and Sen 1948) that rumen microflora possess the ability to decompose oxalate. Evidence supporting this contention has since been obtained (Dodson 1959; Watts 1957, 1959). Recently a bacterium has been isolated (Michael 1959) which occurs in association with the leaves and bulbs of *Oxalis pes-caprae* and has the ability to metabolise oxalate. The object of the work presented here was as follows:—

(i) To determine by in vitro experiments the effect of high levels of oxalate on a number of bacteria isolated in pure culture from the rumen of sheep.

(ii) To examine' the ability of rumen content to decompose oxalate with a view to isolating bacterial species capable of performing this function.

II. EXPERIMENTAL

Twenty-six apparently different strains of rumen bacteria were isolated from a total of 78 sheep slaughtered at Roseworthy throughout the whole of one year. These organisms have not been identified systematically. They have been catalogued on the basis of morphology, cultural characteristics, and biochemical activities. The effect of oxalate on the growth of these organisms was determined by adding 3 per cent. ammonium oxalate to the medium in which they were isolated (O'Halloran 1962). Growth of organisms was determined absorptiometrically at 650 mμ and comparisons were made with growth in the same medium without oxalate, but containing an identical inoculum of the organism under investigation.

Rumen samples were collected aseptically at slaughter. Containers used to collect the rumen content were filled completely, then capped to maintain conditions of low oxygen tension. Samples were processed and inocula taken within one hour of slaughter. Samples were strained through sterile cheese-cloth which had previously been boiled three times in distilled water.

For oxalate decomposition studies one ml of the filtrate and 1 g of the residue were added to 9 ml of the medium described in Table 1.

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TABLE 1

OXALATE ENRICHMENT MEDIUM

Solution A

Potassium oxalate $(COOK)_2$ H_20	5 g
Potassium di-hydrogen phosphate KH_2PO_4	5 g
Sodium thio-glycollate (or Sodium Sulphide Na_2S $9H_20$)	1 g
Ammonium sulphate	1 g
Magnesium sulphate $MgSO_4$ $7H_20$	0·2 g
Ferrous sulphate $FeSO_4$ $7H_2O$	0·05 g
Calcium sulphate $CaSO_4$	0·02 g
Manganese sulphate $MnSO_4$ $4H_2O$	0·002 g
Sodium molybdate $NaMoO_4$ $2H_2O$	0·001 g
Glass distilled water	1 litre

pH 7 ·0 Stand overnight and filter

Sterilize at 15 p.s.i. for 15 minutes

Solution B

Thiamine HC 1	10 mg
Riboflavine	10 mg
Pyridoxine	10 mg
Calcium pantothenate	10 mg
Niacin	10 mg
p. Amino benzoic acid	10 mg
Folic acid	10 mg
Cyano cobalamin (Merk)	200 μgm
Biotin	1 μgm
Glass distilled water	10 ml

pH 6·0

Sterilize at 10 p.s.i. for 15 minutes

Dispense 9 ml amounts of solution A prior to sterilization. Immediately before inoculation add 0·05 ml of Solution B and place in boiling water bath for 15 minutes, allow to cool and inoculate.

Decomposition of oxalate was determined by the method of Baker (1952). All were total oxalate determinations.

Where oxalate decomposition was demonstrated the sample was plated out on tc solid media as described in Table 1, but containing 2 per cent. agar (Bacto-Difco Agar) . Plate cultures were incubated at 38°C for 3 days in both aerobic and micro-aerophilic environments (viz. 10 per cent. CO,, 89 per cent. H_2, 1 per cent. 0,). Colonies developing were picked off and incubated at 38°C in the medium of Quayle and Keech (1959). The ability of the pure culture isolated to decompose oxalate was determined.

TABLE 2

THE PERCENTAGE DECOMPOSITION OF OXALATE BY RUMEN CONTENT

Sample No.	Residue *	Filtrate †
	%	%
RS – 1	19·8	3·6
RS – 2	23·7	1·3
RS – 3	18·9	0
RS – 4	17·6	6·7
RS – 5	28·2	0
RS – 6	51·4	9·3
RS – 7		4·3
RS – 8	36·7	0
RS – 9	4·3 ‡	0
RS – 10	27·9	3·1
RS – 11	73·0	10·2
RS – 12	46·7	5·6

* All determinations are for total oxalate.

† Values quoted are means of duplicate determinations.

‡ Micro-organisms similar to RO-16 could not be isolated from this sample.

III. RESULTS

In vitro studies of the effect of high oxalate levels on growth of 26 strains of rumen bacteria failed to show any significant effect. Of the 26 strains investigated only one effectively decomposed oxalate. On isolation in pure culture this organism was designated RO-16. Preliminary studies have shown RO-16 to be a facultatively aerobic, gram negative, motile rod growing well at temperatures between 38-40°C. When incubated aerobically at 38°C in the medium of Quayle and Keech (1959) oxalate is completely decomposed within 48 hours.

It is apparent from the results in Table 2 that the residues of the rumen contents tested when compared with the corresponding filtrates were much more potent in the destruction of oxalate. The differences between residues and filtrates were highly significant ($P < 0 \cdot 0 1$) .

Thus it appears that the enzymatic principle is associated with the residues rather than the filtrates. In all cases (except RS-9) where decomposition of oxalate has occurred an organism morphologically and culturally similar to RO-16 has been isolated.

The following tentative conclusions have been drawn from these studies:—

(i) High levels of oxalate had no apparent effect on the growth of 26 strains of rumen bacteria studied.

(ii) A facultatively aerobic, gram negative, rod decomposing oxalate has been isolated (RO-16).

(iii) Incubation of the solid and fluid portions of rumen content demonstrated that the solid components were significantly more active in the decomposition of oxalate than the fluid.

(iv) Rumen samples decomposing oxalate have all yielded an organism similar to RO-16.

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