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Ecology of *Rhodococcus coprophilus* and Associated Actinomycetes in Fresh Water and Agricultural Habitats

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SUMMARY

Ecological studies on the recently described nocardioform actinomycete *Rhodococcus coprophilus* have shown that very high numbers of this organism can be isolated from the dung of domesticated herbivores and that growth occurs in this substrate. The coccal survival stage contaminates grass in pastures or hay used during the winter months for fodder, and remains viable after ingestion and passage through the rumen. The excreted organism is washed into streams and rivers and can be isolated in high numbers from stream sediments and lake muds. The ratio of *R. coprophilus* to other actinomycetes in stream water samples should provide a useful index for detecting the presence of dairy farm effluents.

INTRODUCTION

Willoughby's (1969) studies on nocardioform actinomycetes in freshwater habitats were significant for not only did he record the numbers and descriptions of species found in habitats that had been studied in detail by many freshwater biologists, but he also attempted to distinguish between aquatic and terrestrial nocardioforms. One taxon, referred to under the trivial name 'Lspi', was found in high numbers in the mud of the profundal and littoral regions of Blelham Tarn, Cumbria, and its tributary streams. The organism was only recovered from two waterlogged soil samples out of six sites examined near the tarn, and Willoughby concluded that Lspi was probably a 'truly aquatic actinomycete'. Lspi strains isolated by Willoughby and collected from a variety of alternative habitats have now been classified as *Rhodococcus coprophilus* (Rowbotham & Cross, 1976, 1977).

Previously this actinomycete had been found in low numbers on starch casein nitrate agar plates used by Cross & Collins (1966) for isolating Micromonospora species from the water of Blelham Tarn. A further strain (CUB415) was isolated by Dr J. Lacey (Rothamsted Experimental Station, Hertfordshire) on nutrient agar from the dust present in bales of mouldy hay, suggesting that the species might have a wider distribution. However, Johnston & Cross (1976) found *R. coprophilus* to be the most numerous nocardioform in samples of water and mud taken from 14 lakes in the English Lake District and noted that the total number of nocardioforms appeared to be related to the nutrient status of the lake; eutrophic lakes such as Blelham Tarn contained higher numbers than the oligotrophic lakes such as Ennerdale and Wastwater. The high numbers of this hitherto unidentified actinomycete in certain lakes and streams and the possibility that it might be used as an indicator organism of enrichment prompted a more detailed study of its distribution and ecology.

Trials (to be reported elsewhere) showed that a mild heat treatment of water samples or

suspensions of soil, sediments or dung reduced the numbers of non-actinomycete bacteria appearing on isolation plates and increased the recovery of actinomycetes. Colloidal chitin agar was used in the early surveys before the alternative M3 isolation medium was developed. The numbers of *Rhodococcus coprophilus*, Streptomyces species and Micromonospora species were similar on both media but the latter medium, on which *R. coprophilus* colonies were conspicuous within 7 days, significantly aided the enumeration of this organism in a variety of substrates.

METHODS

Isolation media and diluent. Initially a modification of the colloidal chitin agar described by Lingappa & Lockwood (1961, 1962) was used for the isolation of actinomycetes (Cross & Attwell, 1974).

M3 agar medium, developed for the preferential isolation of *R. coprophilus*, contained (in 1 l distilled water): KH_2PO_4, 0·466 g; Na_2HPO_4, 0·732 g; KNO_3, 0·10 g; NaCl, 0·29 g; $MgSO_4.7H_2O$, 0·10 g; $CaCO_3$, 0·02 g; sodium propionate, 0·20 g; $FeSO_4.7H_2O$, 200 μg; $ZnSO_4.7H_2O$, 180 μg; $MnSO.4H_2O$, 20 μg; agar, 18·0 g; cycloheximide, 50 mg; thiamin. HCl, 4·0 mg; pH 7·0. A solution containing cycloheximide and thiamin was sterilized by membrane filtration and added to the autoclaved and cooled agar medium to give the final concentrations specified.

Bennett's medium (Jones, 1949), solidified with agar or as a broth, was used for the growth and maintenance of *R. coprophilus* strains. Quarter-strength Ringer's solution (Oxoid tablets) containing gelatin (0·01 %, w/v), pH 7·0, (Straka & Stokes, 1957) was used as a diluent.

Heat treatment. Samples (2·0 ml) of water, milk or cream in 100 × 12 mm glass tubes sealed with silicone rubber bungs were immersed in a water bath at 55 °C for 6 min before further dilution or plating out. All water samples were stored at 4 °C before heating. Suspensions of grass, dung or soil etc. (1:10, w/v) or of hay (1:50, w/v) were homogenized in diluent before heat treatment. Immediately after heat treatment the samples were mixed on a Vortex mixer (Scientific Industries, Queens Village, New York, U.S.A.) and 0·2 ml was spread on each of five agar plates, which were incubated at 30 °C.

Observation and enumeration of actinomycete colonies on isolation plates. The recommendation of Postgate (1969), of counting 200 to 300 colonies on five plates of the same dilution, was followed where possible.

Colonies were examined using an 8× hand magnifier and their identification was confirmed on a Zeiss Photomicroscope fitted with a Vickers 40× (N.A. 0·57) long working distance (12 mm) wide angled objective lens (Vickers Instruments, York). With this lens it was possible to resolve the fine hyphae and single spores at the margin of Micromonospora colonies and minute fragmenting nocardioform colonies, anywhere on the isolation plate, without the problem of condensation on the objective.

Colonies of *R. coprophilus* on colloidal chitin agar were 0·2 to 3·00 mm in diameter after incubation for at least 3 weeks. They were effuse and asteroidal, pale pink to pale orange and lacked aerial mycelium. On M3 agar they were recognizable microscopically after 24 h, with a hand lens after 4 days and by the naked eye after 5 to 7 days. The 1 to 2·0 mm diam. stellate colonies had a bright orange central papilla and, unlike other members of the '*rhodochrous*' complex, they were firmly anchored to the agar by their substrate hyphae. Other actinomycetes were enumerated after 3 weeks incubation.

Growth and survival of R. coprophilus on grass. Grass seed (*Lolium multiflorum* var.

Westerworlths) was sown on John Innes potting compost no. 1 in plastic seed trays (0·3 × 0·2 m) and germinated in a cold greenhouse. After 3 weeks growth the grass blades were cut to 20 mm to encourage tillering and to thicken the sward. After a further 10 days the grass was about 100 mm high. It was then inoculated by spraying with a washed suspension of *R. coprophilus* (CUB118) in distilled water or a homogenate of fresh cow dung in distilled water (1 : 10, w/v), using a laboratory spray gun (Shandon Scientific Co., London). The trays were watered from below, and, in one experiment, were also sprayed from above with distilled water in the morning and late afternoon to simulate rain and dew. After further growth, the leaf blades from an area approximately 100 × 100 mm were cut off about 10 mm above the level of the compost with sterilized scissors, and the bulked lamina were cut into 5 to 10 mm lengths. One gram of the cut grass was used for dry weight determinations. A further gram was suspended in 9·0 ml diluent, mixed on a Vortex mixer for 2 min, and allowed to stand for 2 min. The supernatant fluid was transferred to a second bottle and, after mixing for 30 s, 2·0 ml of the suspension was heat treated and spread on M3 agar.

Isolation of phage. Dung (10 g) from the surface of old cow pats was homogenized, allowed to stand at room temperature for 1 h, and then the supernatant fluid was centrifuged for 20 min at 1950 ***g***. The resultant khaki liquid was membrane filtered (pore size 0·45 μm) and 6·5 ml was added to a 250 ml flask containing 60 ml Bennett's broth seeded with 10 ml of a 48 h broth culture of *R. coprophilus* CUB687. This flask and a control culture were incubated on a rotary shaker for 5 days at 25 °C and then the turbid orange suspension was spread on Bennett's agar. Lawns were examined for plaques after 2 days incubation at 30 °C.

RESULTS AND DISCUSSION

Rhodococcus coprophilus in stream waters

A number of tributary streams on the north bank of the River Wharfe, Yorkshire, were sampled for *Rhodococcus coprophilus* and associated actinomycetes (Table 1). High numbers of *R. coprophilus* were found in Dean Beck and Riffa Beck in contrast to the low numbers in the River Wharfe, River Washburn, Hundwith Beck and most other streams in which *Micromonospora* was the numerically dominant genus. A survey of Dean and Riffa Becks revealed evidence of organic pollution. Drains from dairy farms discharged into both streams and Sphaerotilus was observed on the stream bed below the drains but was absent upstream of them. Analysis of further samples from Riffa and Dean Becks (Table 2) suggested that the majority of *R. coprophilus* in the lower reaches of these streams originated from the farm drains. These limited studies suggest that low numbers of Micromonospora, Streptomyces and *R. coprophilus*, with an order of frequency M. > S. > R. or M. > R. > S., are found in a typical stream of the Yorkshire Dales. High numbers of actinomycetes, with a very high proportion of *R. coprophilus*, are found in streams polluted by drains from dairy farms.

To determine the origin of *R. coprophilus* strains in Dean Beck, further samples were collected (Fig. 1) and the numbers of actinomycetes were determined (Table 3). Upstream from the drain inflows (sites 1 and 2) the water in the beck was clear, there were caddis fly larvae under the stones and no Sphaerotilus; below the drain inflows the water was turbid and the stream bed was covered by Sphaerotilus. There was a high number of *R. coprophilus* in cow manure collected from the farm and the main source of this organism to Dean Beck was from drain A. The water in this drain resembled a 1 : 100 dilution of cow dung; it was later found to flow from the farm's cow-effluent tank but we were unable to trace the exact source of the second drain.

Table 1. *Numbers of actinomycetes in streams flowing into the River Wharfe, Yorkshire (sampled 16 Nov. 72)*

Sampling site and grid reference*	Numbers (c.f.u. ml^{-1}) on colloidal chitin agar			
	Streptomyces	Micro-monospora	*R. coprophilus*	Totals
Stream, Langbar. SE 087504	45	330	195	570
Dean Beck, Nesfield. SE 094495	140	205	1135	1480
Stream, Ilkley. SE 108485	70	365	75	510
Stream, Middleton. SE 121485	55	210	50	315
Bow Beck, Ben Rhydding. SE 141483	35	235	105	375
Hundwith Beck. SE 153483	25	85	10	120
West Beck, Asquith. SE 167483	50	285	170	505
East Beck, Asquith. SE 170482	40	245	125	410
Stream, Asquith. SE 174475	155	125	55	335
Stream, Weston. SE 175475	30	350	105	485
Stream, Weston. SE 180470	50	215	205	470
Stream, Copmanroyd. SE 207462	80	415	300	795
River Washburn (outflow from Lindley reservoir). SE 232464	20	160	75	255
River Wharfe (upstream of Riffa Beck). SE 257438	10	110	35	155
Riffa Beck, Pool. SE 257459	110	325	1065	1500

* Ordnance Survey, sheet 104, 1:50000 (First Series).

Table 2. *Numbers of actinomycetes in Riffa and Dean Becks and the associated farm drains (sampled 3 Jan. 73)*

Sampling site and grid reference*	Numbers (c.f.u. ml^{-1}) on colloidal chitin agar			
	Streptomyces	Micro-monospora	*R. coprophilus*	Total
Riffa Beck				
Upstream of farm; bordering land is fenced grassland. SE 257467	5	115	10	130
Upstream of farm; bordering land is unfenced cattle pasture, cows have access to the beck. SE 257466	25	175	50	250
Downstream of farm drain inflow; bordering land is fenced cattle pasture, water had a green/khaki tint. SE 257465	55	245	1025	1325
900 m downstream from farm and 10 m above confluence with R. Wharfe. SE 257459	50	80	90	220
Dean Beck				
Open farm drain 1 m before inflow into Dean Beck; water turbid with a green/khaki tint. SE 096496	1150	400	1750	3300

* Ordnance Survey, sheet 104, 1:50000 (First Series).

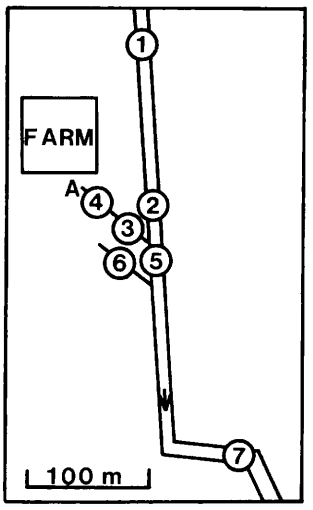

Fig. 1. Diagram to show the location of the seven sampling sites on Dean Beck and the farm drains. Numbers of actinomycetes in the samples are given in Table 3. The arrow indicates the direction of water flow in the main stream.

A sample taken earlier from the stream (Table 1) was from the reach between site 5 and the inflow of the second drain, and the drain sample (Table 2) was from site 3.

Table 3. *Numbers of actinomycetes in Dean Beck and farm drains*

The sampling sites are shown in Fig. 1. The fresh cow dung sample was from the farm: cows were feeding on pasture grass and cattle cake.

	Numbers (c.f.u. ml^{-1}) on M3 agar				
Sample site	Streptomyces	Micro-monospora	*R. coprophilus*	Total	Percentage of *R. coprophilus*
1. Stream	15	$3{\cdot}5\times10^{2}$	95	$4{\cdot}6\times10^{2}$	21
2. Stream	13	$3{\cdot}8\times10^{2}$	88	$4{\cdot}9\times10^{2}$	18
3. Drain A	$5{\cdot}1\times10^{2}$	$3{\cdot}2\times10^{3}$	$1{\cdot}3\times10^{4}$	$1{\cdot}7\times10^{4}$	79
4. Drain A	$8{\cdot}4\times10^{2}$	$3{\cdot}1\times10^{3}$	$1{\cdot}9\times10^{4}$	$2{\cdot}3\times10^{4}$	83
5. Stream	40	$4{\cdot}2\times10^{2}$	$4{\cdot}1\times10^{2}$	$8{\cdot}4\times10^{2}$	48
6. Drain	$1{\cdot}0\times10^{2}$	$8{\cdot}4\times10^{2}$	$8{\cdot}0\times10^{2}$	$1{\cdot}7\times10^{3}$	47
7. Stream	30	$5{\cdot}3\times10^{2}$	$4{\cdot}1\times10^{2}$	$9{\cdot}3\times10^{2}$	43
Farm, fresh cow dung*	$1{\cdot}9\times10^{3}$	$3{\cdot}2\times10^{5}$	$4{\cdot}5\times10^{5}$	$7{\cdot}7\times10^{5}$	58

* Numbers expressed as c.f.u. (g wet wt)$^{-1}$.

Possible sites for the growth of R. coprophilus

Rumen. For an organism to be considered as an active member of the rumen microflora: (i) it must grow under anaerobic conditions at ruminant body temperatures; (ii) the rumen contents should contain not less than 10^{6} of the organism per g wet wt of fresh rumen contents; and (iii) it should produce the type of end product found in the rumen from substrates occurring there (Gall & Huhtanen, 1951).

Rhodococcus coprophilus is a strict aerobe. It has an optimum growth temperature of 31 to 32 °C and a maximum growth temperature of 39 °C when incubated on Bennett's agar slopes in a polythermostat. When isolation plates were incubated at 37 °C instead of 30 °C

Table 4. *Numbers of actinomycetes in samples taken from the bovine rumen and rectum*

Sample	Numbers [c.f.u. (g wet wt)$^{-1}$] on M3 agar		
	Streptomyces	Micromonospora	*R. coprophilus*
Cow 1 (11 Oct. 73)			
Rumen contents	$2{\cdot}0 \times 10^4$	$5{\cdot}4 \times 10^5$	$9{\cdot}0 \times 10^4$
Rectum contents	$5{\cdot}5 \times 10^3$	$3{\cdot}1 \times 10^5$	$4{\cdot}1 \times 10^5$
Cow 2 (16 Oct. 73)			
Rumen contents	$3{\cdot}9 \times 10^4$	$3{\cdot}6 \times 10^4$	$3{\cdot}7 \times 10^4$
Rectum contents	$1{\cdot}1 \times 10^3$	$2{\cdot}0 \times 10^5$	$6{\cdot}8 \times 10^4$

Table 5. *Numbers of Rhodococcus coprophilus in cattle provender and fresh dung*

Sample and source	Numbers [c.f.u. (g wet wt^{-1})] on M3 agar*
Farm A: cows on pasture	
Cattle cake	40
Pasture grass	$1{\cdot}9 \times 10^5$
Fresh cow dung	$4{\cdot}6 \times 10^5$
Farm B: cows indoors (8 Feb. 74)	
Concentrate mixture	50
Seed hay	$2{\cdot}0 \times 10^3$
Fresh cow dung	$9{\cdot}3 \times 10^3$
Farm B: cows indoors, now feeding on meadow hay (12 Mar. 74)	
Meadow hay	approx. 10^6†
Fresh cow dung	$2{\cdot}4 \times 10^6$
Farm C	
Seed hay	50
Farm D	
Ensilage (grass)	25

* Approximately 10 g grass, 7 g fresh dung and 1 g hay is equivalent to 1 g dry material.
† High numbers of Streptomyces in this sample (> 10^7 g^{-1}) prevented the accurate enumeration of *R. coprophilus*.

there was a marked decrease in recovery. Thus the temperatures and conditions in the bovine and ovine rumen are unsuitable for the growth of *R. coprophilus*.

Samples from the rumen and rectum of two cows taken immediately after slaughter contained *R. coprophilus* but the numbers in the rumen did not exceed 10^5 colony-forming units (c.f.u.) (g wet wt)$^{-1}$ (Table 4). The apparent slight increase in numbers in the rectum was probably due to dehydration of the gut contents. Recoveries from both rumen and rectum show that *R. coprophilus*, Micromonospora and Streptomyces can pass through the cow in a viable condition.

Thus *R. coprophilus* does not fulfil two of the three main criteria of Gall & Huhtanen (1951) for it to be an active member of the rumen microflora. It also seems unlikely that an organism capable of using major end products of rumen metabolism, e.g. acetate, propionate and valerate, as sole carbon sources (Rowbotham & Cross, 1977) could produce them as well. Also the organism is not cellulolytic.

Table 6. *Numbers of Rhodococcus coprophilus on various types of grassland*

Site, sample and date	Numbers [c.f.u. (g wet wt)$^{-1}$] on M3 agar
Private house	
Lawn grass (20 Oct. 73)	25
Park	
Permanent grassland (mainly *Holcus mollis*), not manured or grazed (24 Nov. 73)	< 25
Supporting soil (24 Nov. 73)	< 100
Permanent moorland grassland (mainly *Festuca tenuifolia*), not manured or grazed (24 Nov. 73)	< 100
Farm B	
Hay-field grass, new growth, frequently manured (8 Feb. 74)	$3{\cdot}0 \times 10^5$
Sheep pasture, not manured (8 Feb. 74)	$3{\cdot}1 \times 10^4$
Farm A	
Cattle pasture grass (26 Oct. 73)	$1{\cdot}0 \times 10^5$
Farm E	
Cattle pasture grass (4 Nov. 73)	$1{\cdot}3 \times 10^5$
Supporting soil (4 Nov. 73)	$3{\cdot}4 \times 10^5$
Cattle pasture grass (17 Nov. 73)	$1{\cdot}9 \times 10^5$
Supporting soil (17 Nov. 73)	$6{\cdot}6 \times 10^5$
Farm A	
Fresh dung from sheep feeding on pasture grass (26 Oct. 73)	$1{\cdot}1 \times 10^6$

Table 7. *Survival of Rhodococcus coprophilus on grass grown in a greenhouse*

Trays of grass were sprayed with 10 ml of a 1:10 dilution of fresh cow dung containing $1{\cdot}6 \times 10^4$ c.f.u. *R. coprophilus* ml^{-1}. *Rhodococcus coprophilus* could not be recovered from the grass on control trays.

Time after spraying (days)	Numbers of *R. coprophilus* on M3 agar	
	[c.f.u. (g wet wt)$^{-1}$]	[c.f.u. (g dry wt)$^{-1}$]
1	160	$1{\cdot}7 \times 10^3$
7	70	$8{\cdot}5 \times 10^2$
14	< 10	< 100
21	< 10	< 100

If the source of *R. coprophilus* is external, then animals on a diet containing few organisms should only excrete low numbers of them. This could not be put to a direct test although we did study dairy cows fed on pasture grass and concentrates and others, in stalls for the winter, fed on hay and concentrates (Table 5). There were negligible numbers of *R. coprophilus* in cattle concentrate and ensiled grass but high numbers on pasture grass and some hay samples. When in-wintering cows on farm B were fed 'meadow hay' instead of seed hay, the number of *R. coprophilus* in their hay rose to approximately 10^6 c.f.u. (g wet wt)$^{-1}$; the very high numbers of streptomycetes present prevented an accurate enumeration of the nocardioform bacteria. The numbers of *R. coprophilus* in cow dung were in proportion to those in the diet. These studies support the view that *R. coprophilus* strains inside the cow and those excreted by the cow are of external origin. The high numbers on pasture grass indicated that grass might be the site where growth occurred.

Grass. Various samples of grass and the supporting soil were homogenized, heat treated and plated on M3 agar (Table 6). There were high numbers of *R. coprophilus* (10^5 c.f.u. g^{-1})

Table 8. *Survival of Rhodococcus coprophilus on wet grass*

Trays of grass were sprayed with 10 ml of a 1:10 dilution of fresh cow dung (containing $2{\cdot}0 \times 10^5$ c.f.u. *R. coprophilus* ml^{-1}) or with 10 ml of a washed suspension of *R. coprophilus* (CUB118) in distilled water (containing $1{\cdot}2 \times 10^6$ ml^{-1}). Thereafter they were sprayed twice daily with distilled water. There was about 21 g grass on the trays when they were sprayed with *R. coprophilus* and 208 g after 4 weeks, i.e. a 10-fold increase. *Rhodococcus coprophilus* could not be recovered from the grass on control trays.

Time after spraying (days)	Numbers of *R. coprophilus* on M3 agar	
	[c.f.u. (g wet wt)$^{-1}$]	[c.f.u. (g dry wt)$^{-1}$]
With dilute cow dung:		
1	$7{\cdot}9 \times 10^4$	$1{\cdot}0 \times 10^6$
7	$1{\cdot}3 \times 10^4$	$1{\cdot}6 \times 10^5$
14	$1{\cdot}6 \times 10^3$	$2{\cdot}0 \times 10^4$
21	$1{\cdot}6 \times 10^2$	$6{\cdot}0 \times 10^3$
28	< 100	< 1000
With *R. coprophilus* suspension:		
1	$1{\cdot}9 \times 10^4$	$2{\cdot}4 \times 10^5$
7	< 10	< 100

on grass from cattle pastures and the soil beneath; there was a similar number on the new growth of a manured hay field and slightly less on sheep pasture. *Rhodococcus coprophilus* was present, at most, in very low numbers on ungrazed, unmanured pasture or lawn grass. A high number on grass or its supporting soil was therefore associated with the presence of grazing animals or manuring with their dung. The seed hay from farm B (Table 5), which had been grown from seed with the aid of artificial fertilizers and a single application of farmyard manure, carried only low numbers. A sample of seed hay from an alternative source contained less than 50 c.f.u. *R. coprophilus* g^{-1}.

The high number of *R. coprophilus* on the grass from cattle pastures could have been due to its growth on the lamina. In trays of grass sprayed with a 1:10 homogenate of cow dung, the numbers of *R. coprophilus* showed a rapid fall within 14 days (Table 7). This experimental system differed from natural conditions in that there was no dew or rain; the surface of the greenhouse grass was always dry unlike that in the field which is wet for at least part of the day in West Yorkshire. In trays of grass sprayed twice daily with water, the *R. coprophilus* in diluted cow dung survived for longer periods but there was no evidence of growth (Table 8).

Dung. The most probable remaining site for the growth of *R. coprophilus* in the dairy farm environment was dung. Very high numbers were found in samples from old cow pats on pastures (Table 9) compared with the numbers in fresh dung (see Table 5). In order to show that growth could occur on cow dung, as these results inferred, a fresh dung sample voided by a cow on a low *R. coprophilus* diet of seed hay and concentrates was incubated in a glass trough on the laboratory window-sill for 3 weeks. The laboratory temperature was approximately 20 °C during the day falling to a lower temperature during the night and at weekends; the sample was sprinkled with 5 ml sterile distilled water each morning and evening. It was hoped that these incubation conditions simulated those of a cow pat on pasture during spring and summer. The numbers of *R. coprophilus* in the surface layers increased significantly showing that growth had occurred. The studies also showed that the organism in cow dung can survive desiccation and winter weather conditions such as frosts and snow (Table 9).

Table 9. *Numbers of Rhodococcus coprophilus in cow dung and the effect of various environmental conditions*

Sample: source, treatment and date	Numbers of *R. coprophilus* on M3 agar [c.f.u. (g wet wt)$^{-1}$]	[c.f.u. (g dry wt)$^{-1}$]
Old weathered cow pat on pasture (17 Nov. 73)	$8{\cdot}1 \times 10^6$	ND
Old weathered cow pats on pasture (19 Oct. 73) (mixture from 10 pats)	$1{\cdot}8 \times 10^8$	$5{\cdot}7 \times 10^8$
Experiment 1		
Fresh dung from in-wintering cows fed on seed hay and concentrates (20 Feb. 74)	$3{\cdot}0 \times 10^4$	$2{\cdot}0 \times 10^5$
Sample from the same dung after 3 weeks on the laboratory window-sill, watered each morning and afternoon	$3{\cdot}0 \times 10^7$	$3{\cdot}0 \times 10^7$
Experiment 2		
Fresh dung from in-wintering cows fed on meadow hay and concentrates (12 Mar. 74)	$2{\cdot}0 \times 10^6$	$1{\cdot}4 \times 10^7$
Samples from the same dung after:		
6 days at 2 to 4 °C	$1{\cdot}6 \times 10^5$	$1{\cdot}2 \times 10^6$
Drying in the laboratory for 3 weeks	$3{\cdot}0 \times 10^4$	$3{\cdot}0 \times 10^4$
Incubation in a humid chamber at room temperature for 3 weeks	$3{\cdot}6 \times 10^7$	$4{\cdot}4 \times 10^7$
3 weeks on the laboratory window-sill, watered each morning and afternoon	$2{\cdot}3 \times 10^9$	$3{\cdot}4 \times 10^9$
Leaving outdoors on the roof in frost and snow	$9{\cdot}0 \times 10^4$	$1{\cdot}1 \times 10^5$
Experiment 3		
Fresh dung from in-wintering cows fed on hay and concentrates (11 Apr. 74)	$3{\cdot}0 \times 10^3$	$2{\cdot}2 \times 10^4$
Samples from the same dung after drying in the laboratory for 2 weeks, then spraying each evening with distilled water and sampling after:	$2{\cdot}1 \times 10^3$	$2{\cdot}1 \times 10^3$
1 week	$6{\cdot}9 \times 10^6$	$3{\cdot}1 \times 10^7$
2 weeks	$1{\cdot}2 \times 10^7$	$6{\cdot}6 \times 10^7$
3 weeks	$1{\cdot}7 \times 10^7$	$8{\cdot}4 \times 10^7$
4 weeks	$4{\cdot}2 \times 10^6$	$1{\cdot}9 \times 10^7$
Samples from the same dung after drying in the laboratory for:		
4 weeks	$6{\cdot}0 \times 10^2$	$6{\cdot}0 \times 10^2$
6 weeks	$8{\cdot}1 \times 10^2$	$5{\cdot}8 \times 10^2$

ND, Not determined.

Rhodococcus coprophilus was isolated from the dung of other farm and domesticated animals, e.g. horse [$1{\cdot}0 \times 10^3$ c.f.u. (g wet wt)$^{-1}$], donkey [$4{\cdot}0 \times 10^4$ c.f.u. g^{-1}], sheep [$1{\cdot}1 \times 10^6$ c.f.u. g^{-1}] and goat [$4{\cdot}0 \times 10^4$ c.f.u. g^{-1}], which had grazed on pastures previously frequented by cattle. It was also recovered from unpasteurized farm-bottled milk where it accumulated in the cream layer [$1{\cdot}9 \times 10^3$ c.f.u. ml^{-1}].

Phage usually attack growing organisms. Willoughby, Smith & Bradshaw (1972) failed to recover Lspi (*R. coprophilus*) phage from the benthic mud of Blelham Tarn or sediment from Tock How Beck (personal communication) suggesting that the organism was not growing in these habitats. However, we obtained typical phage plaques on lawns derived from broth cultures enriched with filtered dung samples. No protozoa or bacteria were present in the plaques and once the host lawn had grown, no further increase in plaque size occurred. Phage could not be isolated from five soil samples taken from a cow pasture and control plates never exhibited plaques.

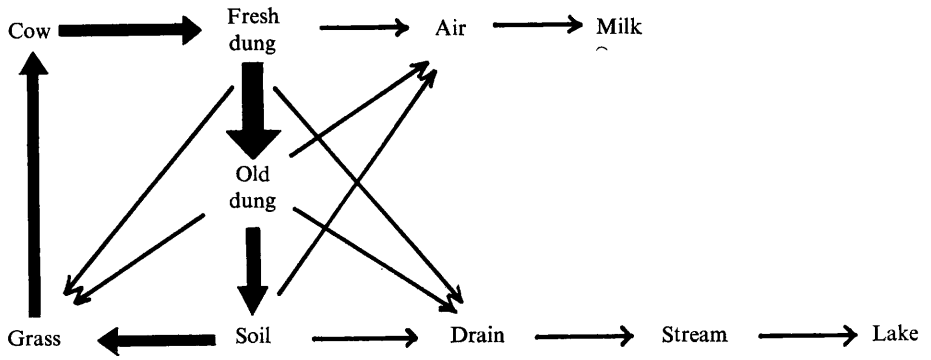

Fig. 2. Scheme to illustrate the cycling and dispersal of *R. coprophilus* in dairy farm and aquatic habitats.

Rhodococcus coprophilus is a common organism in habitats frequented by grazing animals. The coccal survival stage (Rowbotham & Cross, 1977) will contaminate grass and the underlying soil, is washed into streams and rivers and eventually accumulates in the surface muds of lakes (Fig. 2). It represents yet another bacterial species which may prove useful in surveys of potable water supplies for detecting the presence of faecal material of animal origin.

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