INITIAL SURVEY OF ACETYLENE REDUCTION AND SELECTED MICROORGANISMS IN THE FECES OF 19 SPECIES OF MAMMALS

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ABSTRACT.—Nitrogen-fixing bacteria, as demonstrated by the acetylene reduction method; yeasts, and actinomycetes were found in feces of mammals collected from St. Lawrence Island, Alaska, to the North Carolina–Tennessee border. The mammals, representing four orders and 19 species, occupy a wide variety of habitats and may play an important role in dispersing microorganisms vital to the ecosystem.

The California red-backed vole (*Clethrionomys californicus*), the northern flying squirrel (*Glaucomys sabrinus*), and the deer mouse (*Peromyscus maniculatus*) are forest-dwelling rodents that may play an important role in maintaining forest productivity. These mammals consume hypogeous mycorrhizal fungi and disperse fecal pellets containing fungal spores, which germinate and form mycorrhizae with roots of forest trees (Hunt and Maser 1985, Maser et al. 1978; *Food habits*, 1985; *Northern flying squirrel*, 1985; Ure and Maser 1982). The feces of these animals also contain nitrogen-fixing bacteria and yeast. The nutrient in the feces is as effective as yeast extract in promoting bacterial growth and nitrogenase activity (Li et al. 1986). When these animals dig at the bases of trees, the organisms in their feces could inoculate rootlets with nitrogen-fixing bacteria, yeast, and spores of mycorrhizal fungi. At times, actinomycetes are also present; they produce substances important in formation of soil humus (Krassilnikov 1981).

Having worked out the basic links in the abilities of small mammals in western Oregon to pass viable nitrogen-fixing bacteria and yeast through their digestive tracts (Li et al. 1986), the next question was, How widespread is this phenomenon? We conducted a survey of feces of 51 mammals of 19 additional species, collected from St. Lawrence Island, Alaska, to the North Carolina–Tennessee border, for acetylene-reducing (nitrogen-fixing) bacteria (Postgate 1982), yeasts, and actinomycetes.

MATERIALS AND METHODS

Fresh fecal pellets from 51 mammals of 19 species, representing four orders, were collected in sterile vials (see Li and Maser 1986 for collecting techniques).

Acetylene Reduction Activity

One fecal pellet each from 48 small mammals was placed in 20 ml of Döbereiner's N-free liquid medium (Döbereiner and Day 1976) and Burk's (1930) liquid medium in a 60-ml serum bottle. We used only the central portions of the pellets from three large mammals: black-tailed jackrabbit (*Lepus californicus*), eastern cottontail (*Sylvilagus floridanus*), and elk (*Cervus elaphus*). Bottles were capped and flushed for 5 min with nitrogen gas containing less than 10 ppm oxygen. The liquid medium became turbid after incubation for two days at 30 C. Acetylene was then injected into each bottle to 10 percent (v/v); the bottles were gently swirled immediately after acetylene was added and were left to stand at 30 C. Bottles without acetylene injection served as controls. After 18 hr, 0.1 ml gaseous samples were removed from each bottle and analyzed for ethylene and acetylene with a Hewlett-Packard 5830A gas chromatograph[3] fitted with a 2 m × 2.1 mm, 80–100 mesh Poropak R column. Oven temperature was adjusted to 70 C. Injection and flame ionization detector temperatures were each adjusted to 100 C. Nitrogen carrier gas flow rate was adjusted to 40 ml/min.

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TABLE 1. Nitrogenase activity and microorganisms in feces of 51 mammals of 19 species from Alaska to North Carolina–Tennessee.

Animal	Geographic location	Nitrogenase[1] activity	Yeasts[2]	Actinomycetes[2]
INSECTIVORA				
Soricidae (shrews)				
Sorex cinereus (1)[3]	Morocco, IN	+	not determined	not determined
LAGOMORPHA				
Leporidae (hares and rabbits)				
Lepus californicus (1)	Summer Lake, OR	+	+	+
Sylvilagus floridanus (1)	Morocco, IN	+	0	0
RODENTIA				
Sciuridae (squirrels)				
Cynomys leucurus (7)	Meeteetse, WY	0	+	0
Cynomys leucurus (3)	Laramie, WY	0	+	0
Eutamias townsendi (1)	Umpqua, OR	+	+	+
Glaucomys volans (2)	Morocco, IN	+	+	0
Spermophilus parryi (1)	St. Lawrence Island, AK	0	+	0
S. tridecemlineatus (2)	Cedar Falls, IA	+	+	+
Tamias striatus (1)	Cedar Falls, IA	+	+	+
Geomyidae (pocket gophers)				
Geomys busarius (1)	Morocco, IN	+	0	+
Cricetidae (native mice)				
Peromyscus leucopus (6)	Morocco, IN	+	+	0
P. maniculatus (4)	Morocco, IN	+	0	0
Arvicolidae (voles)				
Clethrionomys gapperi (10)	Roon Mountain, NC–TN	+	+	+
Lagurus curtatus (1)	Silver Lake, OR	+	+	+
Microtus ochrogaster (2)	Morocco, IN	+	+	0
M. oeconomus (4)	St. Lawrence Island, AK	+	+	0
M. oregoni (1)	Marys Peak, OR	+	+	+
Muridae (Old World mice)				
Mus musculus (1)	Morocco, IN	+	+	+
ARTIODACTYLA				
Cervidae (deer)				
Cervus elaphus (1)	Post, OR	0	+	0

[1]Four replicates (all positive or all negative).
[2]Average of three replications of one individual from each species.
[3]Number of individuals tested for nitrogenase activity.

Bacterial cultures that reduced acetylene were all isolated but were not all purified.

Yeast and Actinomycetes

Sodium albumenate agar (Waksman and Fred 1922) was used to test for yeast and actinomycete populations. One fecal pellet (or central portion of a pellet from the three large mammals) per vial was removed with sterile forceps under an isolation hood. Each pellet was crushed and thoroughly mixed in 30 ml of sterile distilled water. One ml, 0.5 ml, and 0.1 ml of this fecal suspension were each plated with 20 ml of sodium albumenate agar. Colonies developed on the surface of the agar. The presence of yeasts and actinomycetes was confirmed under a light microscope, and colonies were counted after three days' incubation at 30 C. Colonies were sometimes so numerous, even at high dilutions, that their numbers had to be estimated.

RESULTS AND DISCUSSION

Results of our study are given in Table 1.

Acetylene Reduction Activity

Feces of mammals of the 19 species were tested for acetylene reduction, which is a universal and specific property of nitrogenase of nitrogen-fixing bacteria (Postgate 1982). Sixteen samples were positive (Table 1). Thirteen of the 19 species are known to eat the fruiting bodies of hypogeous, mycorrhizal fungi from which they could ingest nitrogen-fixing bacteria (Li and Castellano 1985, 1986). These 13 mycophagists are: masked shrew (*Sorex cinereus*) (Hamilton 1941); *Lepus cali-*

fornicus (Ponder 1980); Townsend chipmunk (*Eutamias townsendi*) (Maser et al. 1978); southern flying squirrel (*Glaucomys volans*) (Maser and Maser, unpublished data); eastern chipmunk (*Tamias striatus*) (Maser and Maser, unpublished data); white-footed mouse (*Peromyscus leucopus*) (Fogel and Trappe 1978, Maser et al. 1978, Whitaker 1966); *P. maniculatus* (Hunt and Maser 1985, Maser et al. 1978); southern red-backed vole (*Clethrionomys gapperi*) (Fogel and Trappe 1978, Maser et al. 1978, Ure and Maser 1982); sage vole (*Lagurus curtatus*) (Dowding 1955, Maser et al. 1978); prairie vole (*Microtus ochrogastar*) (Fogel and Trappe 1978); tundra vole (*M. eoconomus*) (Fogel and Trappe 1978); creeping vole (*M. oregoni*) (Maser et al. 1978); and house mouse (*Mus musculus*) (Whitaker 1966).

Yeast

Mammals that feed on fungi can also ingest yeasts (Anderson and Skinner 1947, Kockova-Kratochvilova et al. 1984). Yeasts were virtually ubiquitous in our samples and passed through the digestive tracts of 15 of the 18 species checked (Table 1). Yeast propagules ranged from zero to an estimated 1,800,000 per pellet.

Actinomycetes

Actinomycetes, often called "ray fungi," are actually higher bacteria. They may occur both in soil and on the surfaces of plant leaves (Dickinson et al. 1975, Lechevalier 1981). Of the 18 species examined for actinomycetes, the 9 that were positive (Table 1) are known to eat substantial amounts of green vegetation (Bailey 1936, Bee and Hall 1956, Hamilton and Whitaker 1979, Hansen and Flinders 1969, Lechleitner 1969, Whitaker 1966). Actinomycetes ranged from zero to an estimated 600,000 per fecal pellet.

Potential Interrelations

Mammals generally are abundant and mobile and form functional links with all areas of the terrestrial habitat, from below the ground into the tree tops. They deposit fecal pellets throughout their habitats. Fecal pellets of some species contain viable nitrogen-fixing bacteria, yeast propagules, and actinomycetes (Table 1). Yeast and actinomycetes apparently can be obtained by mammals on plant material (Dickinson et al. 1975, Lechevalier 1981); however, nitrogen-fixing bacteria in pellets seem to be associated more with soil and food obtained below the ground. For example, feces of white-tailed prairie dog (*Cynomys leucurus*), arctic ground squirrel (*Spermophilus parryi*), and *Cervus elaphus* showed no acetylene reduction activity (Table 1); their sole diet in early summer when the pellets were collected might have been only the aboveground portions of green vegetation. At other times of the year, their droppings may include nitrogen-fixing bacteria because of shifts in food habits. *Spermophilus parryi*, for instance, may eat mushrooms at certain times (Bee and Hall 1956) and thus may ingest nitrogen-fixing bacteria. *Cervus elaphus* eat hypogeous fungi part of the year (Trappe et al., unpublished data) and may ingest nitrogen-fixing bacteria. Other species, such as the plains pocket gopher (*Geomys bursarius*), feed on subterranean portions of plants and also ingest some soil while digging (Hamilton and Whitaker 1979); this behavior could also account for nitrogen-fixing bacteria in the feces.

Thus far, we have been able to identify only three of the isolated nitrogen-fixing bacteria: *Azospirillum* sp., *Clostridium butyricum*, and *C. beijerinckii*. *Azospirillum* sp. has been isolated from feces of the California red-backed vole and the northern flying squirrel (Li et al. 1986), and the creeping vole (*Microtus oregoni*) (Li and Maser, unpublished data). *Clostridium butyricum* has been isolated from feces of the deer mouse (Li et al. 1986), and *C. beijerinckii* from feces of the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) (this study). These three species of nitrogen-fixing bacteria occur freely in the soil (Buchanan and Gibbons 1974, Hammann and Ottow 1976, Jones and Bangs 1985, Lakshmi et al. 1977). *Azospirillum* sp. can penetrate plant roots (Lakshmi et al. 1977, Patriquin and Döbereiner 1978), and *Azospirillum* sp. and *Clostridium* sp. have also been found associated with ectomycorrhizae of Douglas-fir (*Pseudotsuga menziesii*) (Li and Hung, Plant and Soil, in press).

Some species of yeast may increase nitrogen fixation in the presence of mycorrhizal fungi and thereby improve site productivity (Li et al. 1986, Maser et al. 1984). Yeast in the feces of mycophagous mammals may also be

important because spore germination of some mycorrhizal-forming fungi is stimulated by extractives from other fungi, such as yeast (Fries 1966, 1982, Oort 1974).

Actinomycetes produce substances that are important in the formation of soil humus (Krassilnikov 1981), and humus, in turn, is important to the formation of mycorrhizae (Harvey et al. 1976, Kumuda et al. 1961, Maser et al. 1984). As stated by Linderman (1985), however, microbial interactions are complex, and actinomycetes are but a fraction of the complexity.

Mammals may play an important functional role in dispersing microorganisms vital to the ecosystem. Their potential importance is further suggested when these new data are coupled with the role of mammals in the dispersal of viable spores of mycorrhizal fungi, which are obligatory symbionts of most plants (Fogel and Trappe 1978, Kotter and Farentinos 1984, Maser et al. 1978, Rothwell and Holt 1978, Trappe and Maser 1977).

CONCLUSION

We reiterate that our survey was intended to ascertain the potential geographical scope of nitrogen-fixing bacteria (through acetylene reduction) in mammals. Our survey has some obvious deficiencies: for example, mammals were collected at different seasons and in different habitats, so standardizing was impossible. Isolating and identifying the nitrogen-fixing bacteria was extremely difficult; to our knowledge, no one has done this type of study before and laboratory techniques had to be modified (Li and Maser 1986). Finally, other microorganisms, such as yeasts and actinomycetes, are both poorly known and understood.

To study and understand the vast array of ecosystem processes require a carefully planned, interdisciplinary approach. As we learn more about mammal-habitat interactions, research must be aimed at the mammals as complex, functional links in the ecosystem. Understanding these dynamic linkages will help us to manage wisely both the mammals and their habitats to maintain or improve the health of the ecosystem.

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