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Author: Botzler, R. G.

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Isolation of *Yersinia enterocolitica* and *Y. frederiksenii* from Forest Soil, Federal Republic of Germany

R. G. Botzler, Department of Wildlife, Humboldt State University, Arcata, California 95521, USA

ABSTRACT: This is the first report of *Yersinia frederiksenii* from soil in Europe and of *Yersinia enterocolitica* sensu stricto from soil in the Federal Republic of Germany. These organisms were isolated from deciduous forest soil, but were not found in grassland soils.

Key words: Yersinia enterocolitica, Yersinia frederiksenii, Federal Republic of Germany, soil microbiology.

The *Yersinia enterocolitica* complex is a heterogeneous collection of bacteria that can cause mortality of humans, domestic animals and wildlife. Based primarily on carbohydrate fermentation patterns and DNA homology studies, this complex of bacteria has recently been divided into several species: *Yersinia enterocolitica* sensu stricto, *Y. frederiksenii, Y. intermedia* and *Y. kristensenii* (Brenner et al., 1980).

In recent years these bacteria have been isolated from a variety of environmental sources (Botzler et al., 1968; Kapperud, 1977; Bercovier et al., 1978; Alonso et al., 1979; Agbalika et al., 1985), but their enzootiology, including the identity of their natural reservoirs, is not yet fully understood. Some of these bacteria commonly occur in aquatic ecosystems while others are associated more frequently with terrestrial ecosystems (Kapperud, 1981).

In the USA, some environmental strains of the *Y. enterocolitica* complex have been associated with deciduous forests. In soils of northern California *Yersinia enterocolitica* sensu stricto was isolated more frequently from a red alder (*Alnus rubra*) community than from grasslands (Botzler, 1979). Further, a retrospective analysis of earlier work conducted in southeastern Michigan (Botzler et al., 1976), indicated that water samples and aquatic invertebrates harboring *Y. enterocolitica* sensu stricto and *Y. intermedia* were collected adjacent to an oak-hickory (*Quercus* spp.–*Carya* spp.) forest, whereas these yersinia rarely were found in samples collected adjacent to grasslands. It was speculated that soil of deciduous forests might be the natural habitat for some yersinia.

When the opportunity arose to study these bacteria in the Federal Republic of Germany, it was hypothesized that yersinia would be found in German soils, and would occur more frequently in soils of deciduous forests than in grasslands.

Soil was sampled from four study areas on or near the University of Hohenheim, Stuttgart, Federal Republic of Germany. Two to three samples were taken at each site on a weekly basis in November and December 1981.

Twenty samples were analyzed from deciduous forest soils. Eighteen samples were collected from an even-aged oak-hornbeam (*Quercus robur–Carpinus betulus*) community in the Häslach Woods. Two additional soil samples were taken from a tended, mixed deciduous woodlot of the Hohenheim Schlosspark.

Samples were evaluated from three grassland sites. Twelve samples were taken from a tended grassland plot with planted shrubbery in the Hohenheim Botanical Gardens. Another 12 samples were collected from a sheep pasture adjacent to the Botanical Gardens; the area was not used by sheep during the study period. An additional 18 samples were taken from an ungrazed grass plot adjacent to the oak-hornbeam woodlot of Häslach.

For each sample, a 2–3 g plug of surface

TABLE 1. Variation among the yersinia isolated in November and December 1981 from soil at the University of Hohenheim, Stuttgart, Federal Republic of Germany.

Characteristics	Oak-hornbeam woodlot samples						Schlosspark sample
	W1		W3		W8		S2
Date collected	27 Nov		4 Dec		11 Dec		27 Nov
Yersinia present	Yf[a]	Yf	Yf	Yf	Yf	Ye[b]	Ye
Serovar O:	ns[c]	ns	ns	ns	ns	ns	6,30
Indol	+	+	+	+	+	−	+
Acetylmethylcarbinol	−	+	−	+	−	−	−
Rhamnose	+	+	+	+	+	−	−

[a] *Yersinia frederiksenii*.
[b] *Yersinia enterocolitica*.
[c] Not serotypable with absorbed sera O-factors 1–34, according to Wauters scheme.

soil was collected and stored for 5 mo at 4 C in 9 ml trypticase soy broth with 0.5% yeast extract. Care was taken to avoid sampling any soil in an area with evidence of animal feces. MacConkey agar was used for initial isolations. Suspect colonies were characterized by methods outlined in Cowan and Steel (1965). A selection of isolates was evaluated by Prof. Dr. Med. W. Knapp, University of Erlangen-Nürnberg with antisera for *Y. enterocolitica* sera O-factors 1–34, according to Wauters scheme.

Yersinia were isolated from four (6.5%) of 62 soil samples collected in November and December 1981. *Yersinia frederiksenii* was isolated from three (W1, W3, W8) of 18 soil samples from the oak-hornbeam forest (Table 1). A nonserotypable biotype 3 *Yersinia enterocolitica* also was isolated from one (W8) of the oak-hornbeam soil samples containing *Y. frederiksenii*. A serovar 0:6,30 biotype 1 *Y. enterocolitica* sensu stricto was isolated from one (S2) of two soil samples from the artificial woodlot in the Schlosspark (Table 1).

All of the isolates were gram negative rods, motile at 22 C and nonmotile at 37 C. All were positive for catalase, urease, ONPG, nitrate reductase, and ornithine decarboxylase. All were negative for oxidase, lysine decarboxylase and phenylalanine deaminase. On Kligler iron agar all produced an alkaline slant and acid butt; none produced H_2S in 7 days. With the exception of the serovar 0:6,30 *Y. enterocolitica* which was lost in transit to the USA and could not be evaluated, the isolates also were Simmons citrate positive, but negative for arginine dihydrolase and gelatinase.

All strains fermented glucose in the O-F test, and most strains produced a small bubble of gas in glucose. All of the strains utilized arabinose, fructose, sucrose, soluble starch, salicin, mannitol, sorbitol and inositol in 24 hr; all used xylose, cellobiose and glycerol in 48 hr. None used melezitose or raffinose in 14 days. With the exception of the serovar 0:6,30 *Y. enterocolitica*, the remaining isolates were positive for lactose and amygdalin, but negative for melibiose. Variation occurred among the isolates with tests for indol, acetylmethylcarbinol and rhamnose (Table 1).

Overall, yersinia were isolated from four of 20 deciduous forest soil samples, and none of 42 grassland soil samples in this study. With a chi-square test, this difference was significant ($P < 0.05$).

This is the first known report of *Yersinia frederiksenii* from soil in Europe. It is also the first known report of *Y. enterocolitica* sensu stricto from soil in the Federal Republic of Germany. This species has been reported previously from soil in France (Barré et al., 1976).

The finding of a biotype 1 *Y. entero-*

colitica in soil supports past evidence that biotype 1 strains commonly are associated with terrestrial ecosystems in Europe (Bercovier et al., 1978; Kapperud, 1981). It is of interest to note that a serovar 0:6,30 *Yersinia enterocolitica*, which was isolated from soil in this study, previously had been isolated from humans (Kapperud, 1981).

Schindler (1984) argued that *Yersinia enterocolitica* is not a saprophytic soil bacterium. However, the data from this study support other evidence (Barré et al., 1976; Botzler, 1979) that some members of the *Yersinia enterocolitica* complex may occur regularly in soil. In northern temperate regions these yersinia appear to be more prevalent in soils of deciduous forests than in those of grasslands.

These findings give additional insights on the possible role of the natural environment as a reservoir of yersinia. Thus, they will contribute to developing a model for the enzoology of these bacteria which can be used to predict the best means of preventing the occurrence of these pathogens in wildlife, domestic animals and humans.

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