Taxonomic Study of *Xenorhabdus*, a Genus of Bacteria Symbiotically Associated with Insect Pathogenic Nematodes

RAYMOND J. AKHURST

Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Hobart 7000, Tasmania, Australia

The taxonomy of the bacteria symbiotically associated with the insect-pathogenic nematodes *Neoaplectana* and *Heterorhabditis* was examined. The bacteria studied were isolated from 33 populations obtained from Australasia, Europe, and the United States. The symbionts of all species of *Neoaplectana* and *Heterorhabditis* examined were members of the genus *Xenorhabdus*, but they differed in several respects from the description of the genus *Xenorhabdus*, including the guanine-plus-cytosine content of the deoxyribonucleic acid and the production of acid from carbohydrates. All bacteria isolated from *Heterorhabditis* spp. were identified as members of *Xenorhabdus luminescens*. The bacteria isolated from one *Neoaplectana* species were similar and were distinguishable from the bacteria isolated from other *Neoaplectana* species. The following three subspecies of *Xenorhabdus nematophilus* are proposed: *Xenorhabdus nematophilus* subsp. *nematophilus* (bacteria symbiotic with *Neoaplectana feltiae* [= *Neoaplectana carpocapsae*]; type strain ATCC 19061), *Xenorhabdus nematophilus* subsp. *bovienii* (bacteria symbiotic with *Neoaplectana bibionis*; type strain, UQM 2210^T), and *Xenorhabdus nematophilus* subsp. *poinarii* (bacteria symbiotic with *Neoaplectana glaseri*; type strain, UQM 2216). These subspecies vary in host nematode, pigmentation, maximum temperature for growth, responses to tests for phenylalanine deaminase by secondary-form isolates and for lipase (Tween 80 test) and lecithinase by primary-form isolates, and coloration of primary-form isolates on MacConkey agar and media containing bromthymol blue.

Specific associations between insect-pathogenic nematodes and bacteria have been described for two genera of nematodes. An association between *Neoaplectana* and bacteria was suggested by Bovien (5) for *Neoaplectana bibionis* Bovien and was demonstrated for *Neoaplectana feltiae* Filipjev (synonym, *Neoaplectana carpocapsae* [24]) and for *Neoaplectana glaseri* Steiner by Dutky (S. R. Dutky, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1937) and by Poinar and Brooks (16), respectively. Poinar (15) and Khan et al. (12) showed that *Heterorhabditis* spp. are specifically associated with bacteria.

The infective stages of *Neoaplectana* and *Heterorhabditis* are free-living, nonfeeding juveniles which carry the symbiotic bacteria monoxenically in their guts. After entering an insect host, a nematode moves to the haemocoel, where it releases symbiotic bacteria. The bacteria proliferate, kill the insect, and establish suitable conditions for reproduction of the nematodes by providing nutrients and inhibiting the growth of other microorganisms (18). In due course, a new generation of infective-stage nematodes is produced, and these nematodes pro-

vide the means for the symbiotic bacteria to reach new hosts.

Poinar and Thomas (17) described the bacterial symbiont of *N. feltiae* strain DD136 as a new species, "*Achromobacter nematophilus*." However, when the genus *Achromobacter* was rejected (6), "*A. nematophilus*" could not be assigned to any accepted genus. The bacterial symbionts of two *Heterorhabditis* species were characterized, but not named, by Poinar et al. (19) and Khan and Brooks (11). Subsequently, Thomas and Poinar (25) erected the new genus *Xenorhabdus* within the family *Enterobacteriaceae*, to accommodate the symbionts of *Neoaplectana* and *Heterorhabditis*. These authors described the following two species in this genus: *Xenorhabdus nematophilus* (Poinar and Thomas) Thomas and Poinar and *Xenorhabdus luminescens* Thomas and Poinar. These two species are symbiotic with *Neoaplectana* spp. and *Heterorhabditis* spp., respectively.

Akhurst (1) found that bacterial symbionts of *Neoaplectana* and *Heterorhabditis* produced two colony forms. In general, only one form, designated the primary form, could be isolated from infective-stage nematodes. The secondary

form was isolated from infected insects, from monoxenic in vitro cultures of nematodes and symbiotic bacteria, or from pure cultures of bacterium. The primary form was unstable and was readily converted to the secondary form, which was usually stable. The two forms of *X. nematophilus* were equally pathogenic but differed in their reactions to tests for phenylalanine deaminase, lipase, and the absorption of bromthymol blue from agar media. In in vivo and in vitro cultures the nematodes reproduced more rapidly and more prolifically with the primary form of their *Xenorhabdus* sp. symbiont than with the secondary form.

In this paper I present the results of a taxonomic study of the bacteria isolated from several species of *Neoalectana* and *Heterorhabditis*. Three subspecies of *X. nematophilus* are proposed.

MATERIALS AND METHODS

Bacterial strains. The sources of the nematodes from which the bacteria were isolated are listed in Table 1. Nematode species belonging to the genus *Neoalectana* were identified by cross-breeding (2).

With two exceptions, the primary forms of the bacteria were isolated directly from the infective stages of the nematodes by maceration (1). The symbiont of *N. feltiae* strain DD136 was obtained from the American Type Culture Collection (ATCC 19061^T). The secondary forms of *Xenorhabdus* isolates were obtained either from infected *Galleria mellonella* (L.) larvae or from monoxenic cultures on artificial media.

Stock cultures of the bacteria were maintained on the yeast extract salts agar of Dye (9) (0.5 g of $\text{NH}_4\text{H}_2\text{PO}_4$, 0.5 g of K_2HPO_4 , 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0 g of NaCl, 5.0 g of yeast extract, and 12 g of purified agar in 1 liter of water) at 12°C and subcultured monthly.

Methods. All tests except those requiring shaking were conducted at 28°C; the tests requiring shaking were conducted at 25°C. In general, test media were inoculated by using cells from 1- to 6-day-old YDC agar (10 g of yeast extract, 5 g of dextrose, 20 g of CaCO_3 , and 15 g of agar in 1 liter of water) (9) cultures, which produced considerably higher numbers of cells than cultures in nutrient agar (NA; Difco Laboratories) or yeast extract salts agar. For tests in which growth was examined, the medium was inoculated with a loopful of an aqueous suspension of cells from a YDC agar culture.

Average cell size was estimated by measuring 50 cells from 24-h-old yeast extract salts broth (YSB; yeast extract salts agar without agar) cultures in wet mounts. Motility was assessed by examining hanging drops of 24-h-old YSB cultures, and the flagellum position was determined by transmission electron microscopy after negatively staining cells from 24-h-old YSB cultures. Air-dried films of 24-h-old YSB cultures were stained for the Gram reaction and were checked by treating a heavy suspension of cells from a YSB culture with 10% sodium dodecyl sulfate (10).

Colony and cultural characteristics were studied on NA, tergitol-7 agar (Difco) supplemented with triphen-

yltetrazolium chloride (TTC), MacConkey agar (Difco), Simmons citrate agar (Difco), and triple sugar iron agar (Difco). Pigmentation of colonies was recorded after growth on NA and YDC agar.

Catalase activity was tested by flooding 24-h-old NA cultures with 10% (vol/vol) hydrogen peroxide and also by placing a loopful of cells from a 24-h-old NA culture into a drop of 10% (vol/vol) hydrogen peroxide on a glass slide. These tests were repeated with cultures grown on the GYCA medium of Dye (9) (5 g of glucose, 5 g of yeast extract, 40 g of CaCO_3 , and 15 g of agar in 1 liter of water). The following tests were conducted as described by Dye (9): oxidation-fermentation, oxidase, hydrolysis of starch and esculin, methyl red, nitrate reduction, urease, KCN tolerance, maximum temperature for growth, reducing substances from sucrose, growth factor requirements, and utilization of organic acids (using OY medium). Glucanate utilization was also tested by the method of Shaw and Clarke (22). Acetoin production in shake cultures was tested in the acetoin medium of Dye (9) and in MR-VP medium (Difco) after 2 and 5 days of incubation, as described by Dye (9). Samples from shaken cultures in the indole medium of Dye (9) were tested after 2 and 5 days with Kovac reagent and also with Ehrlich reagent after xylene was added. Casein hydrolysis was tested on Dye OY agar (9) containing 10% (vol/vol) skim milk.

The tyrosinase and chitinase tests used were those used by Khan and Brooks (11). Lecithinase and lipase were tested on yeast extract salts agar containing 5% (vol/vol) fresh egg yolk emulsion (20% [wt/wt] egg yolk in distilled water) and on tryptone agar (11.0 g of tryptone and 12.0 g of agar in 1 liter of distilled water) containing 5% (vol/vol) fresh egg yolk emulsion. Lipase activity was also assessed on the medium of Sierra (23) with the Tween 80 concentration reduced to 0.2% (vol/vol) to allow more vigorous growth. Gelatin hydrolysis was tested on nutrient gelatin, and protease activity was tested on Loeffler blood serum and egg albumin agar (Trypticase soy agar supplemented with 0.11% [vol/vol] CaCl_2 and 1.25% [vol/vol] egg albumin).

The tests of Moeller (13) for arginine dihydrolase and lysine, ornithine, and glutamic acid decarboxylases were used. Deoxyribonuclease activity was tested as described previously (8), cytochrome oxidase was tested by the method of Schaeffer (20), and peroxidase was tested by the method of Anderson (3). The test for phosphatase was conducted on phenolphthalein phosphate agar as described by Cowan and Steel (7). Cultures on phenylalanine agar (Difco) were tested with freshly prepared reagents (8) after 2, 5, and 7 days to assess phenylalanine deaminase activity.

The production of acid from all carbon sources except esculin was assessed in 1% (wt/vol) peptone water containing bromocresol purple and 1% (wt/vol) carbon source; esculin was tested at a concentration of 0.1% (wt/vol). Esculin, inulin, and salicin were tyndallized in the medium; all other carbon sources were filter sterilized and added to the cooled, autoclaved basal medium.

Bioluminescence was determined by examining 48-h-old NA cultures for 10 min in total darkness.

The buoyant densities of the deoxyribonucleic acids (DNAs) of seven isolates were determined jointly by G. Skyring and E. Dennis, using ultracentrifugation

TABLE 1. Sources of the bacterial isolates used in this study

Group	Isolate	Form(s) ^a	Nematode	Source of nematode ^b
I	A24	P	<i>N. feltiae</i> strain Agriotos ^c	Poinar
	A25	S	<i>N. feltiae</i> strain Agriotos	Poinar
	AN/5	P	<i>N. feltiae</i> strain DD136	ATCC 19061
	N55	P	<i>N. feltiae</i>	Murrumbateman, New South Wales, Australia ^d
	Pi	P	<i>N. feltiae</i> strain Pieridarum	Stanuszek
	TN6	P + S	<i>N. feltiae</i> strain Nelson	<i>Vespsula</i> sp., Hobart, Tasmania, Australia
II	TP7	P	<i>N. feltiae</i>	Powranna, Tasmania, Australia ^d
	NBC	P	<i>N. bibionis</i>	Mráček
	N51	P	<i>N. bibionis</i>	Murrumbateman, New South Wales, Australia ^d
	NZ	P	<i>N. bibionis</i>	Wouts
	T228	P	<i>N. bibionis</i>	Dover, Tasmania, Australia ^d
	T231	P	<i>N. bibionis</i>	Risdon Vale, Tasmania, Australia ^d
	T268	P	<i>N. bibionis</i>	Dover, Tasmania, Australia ^d
	T292	P	<i>N. bibionis</i>	Nive River, Tasmania, Australia ^d
	T298	P	<i>N. bibionis</i>	Plenty, Tasmania, Australia ^d
	T302	P	<i>N. bibionis</i>	Bruny Island, Tasmania, Australia ^d
	T307	P	<i>N. bibionis</i>	Bruny Island, Tasmania, Australia ^d
	T319	P	<i>N. bibionis</i>	Mt. Wellington, Tasmania, Australia ^d
	T335/1	P	<i>N. bibionis</i>	<i>Otiorynchus sulcatus</i> , Nicholls Rivulet, Tasmania, Australia
	T335/2	S	<i>N. bibionis</i>	<i>Otiorynchus sulcatus</i> , Nicholls Rivulet, Tasmania, Australia
	V1	P	<i>N. bibionis</i>	Sexton
	V3	P	<i>N. bibionis</i>	Sexton
III	G/1	P	<i>N. glaseri</i>	Kaya
	G/2	S	<i>N. glaseri</i>	Kaya
IV	N37	?	<i>Neoaplectana</i> sp. N (undescribed)	Coonabarabran, New South Wales, Australia ^d
	T80	?	<i>Neoaplectana</i> sp. M (undescribed)	Tonganah, Tasmania, Australia ^d
	T171	?	<i>Neoaplectana</i> sp. M (undescribed)	Cleveland, Tasmania, Australia ^d
	T300	?	<i>Neoaplectana</i> sp. M (undescribed)	Bruny Island, Tasmania, Australia ^d
V	B	P	<i>Heterorhabditis bacteriophora</i>	Wouts
	C	P	<i>Heterorhabditis heliothidis</i>	Wouts
	D	P	<i>Heterorhabditis</i> sp.	Darwin, Northern Territory, Australia ^d
	HP/1	P	<i>Heterorhabditis</i> sp.	Stanuszek
	HP/2	S	<i>Heterorhabditis</i> sp.	Stanuszek
	NZH	P	<i>H. heliothidis</i>	Wouts
	T280/1	P	<i>Heterorhabditis</i> sp.	Wynyard, Tasmania, Australia ^d
	T280/2	S	<i>Heterorhabditis</i> sp.	Wynyard, Tasmania, Australia ^d
	T301	P	<i>Heterorhabditis</i> sp.	Bruny Island, Tasmania, Australia ^d
	T310	P	<i>Heterorhabditis</i> sp.	Sandy Bay, Tasmania, Australia ^d

^a P, Primary form; S, secondary form.^b Poinar, G. O. Poinar, University of California, Berkeley; Stanuszek, S. Stanuszek, Institute of Ecology, Warsaw, Poland; Mráček, Z. Mráček, Entomological Institute, CSAV, České Budějovice, Czechoslovakia; Wouts, W. Wouts, DSIR, Department of Scientific and Industrial Research, New Zealand; Sexton, S. Sexton, Plant Research Institute, Burnley, Victoria, Australia; Kaya, H. Kaya, University of California, Davis.^c *N. feltiae* = *N. carpocapsae*.^d Isolated from soil by the method of Bedding and Akhurst (4).

TABLE 2. Variable characteristics of five groups of strains of *Xenorhabdus*

Characteristic	Group I	Group II	Group III	Group IV	Group V
Host nematode	<i>N. feltiae</i> (= <i>N. car-pocapsae</i>) 7	<i>N. bibionis</i>	<i>N. glaseri</i>	<i>Neoapectana</i> sp. strain M or N	<i>Heterorhabditis</i> spp.
No. of isolates		15	2	4	10
Mean cell length (range)	6.1 (3.1–21.9)	5.8 (2.3–21.9)	4.5 (2.3–9.4)	5.7 (2.3–21.9)	5.9 (2.3–20.3)
Mean cell width (range)	1.1 (0.5–1.6)	1.0 (0.3–1.6)	1.2 (0.8–1.9)	1.1 (0.5–1.6)	1.0 (0.3–1.6)
Pigmentation	Buff	Yellow	Brown	Brown	Yellow-red
Catalase	–	–	–	–	+
Bioluminescence	–	–	–	–	+
Urease	–	–	–	–	+
Esculin hydrolysis	–	–	–	+	+
Lipase (Tween 80)	2+, 5–	+	+	+	+
Phenylalanine deaminase	1+, 6–	–	–	–	5+, 5–
Growth at 34°C	+	–	+	+	5+, 5–
36°C	–	–	+	3–, 1+ ^a	1+, 9–
40°C	–	–	+	–	–
Indole	–	–	–	–	3+, 7–
Phosphatase	–	1+, 14–	–	+	+
Lecithinase	6+, 1–	14+, 1–	–	–	8+, 2–
MacConkey agar: red colonies	–	–	1+, 1–	–	7+, 3–
BTB absorption	6+, 1–	14+, 1–	–	–	9+, 1–
Utilization of: gluconate	4+, 3–	+	+	+	+
propionate	+	11+, 4–	1+, 1–	+	+

^a Isolate of *Neoapectana* sp. strain N symbiont.

and *Micrococcus luteus* DNA as the standard. The guanine-plus-cytosine (G+C) contents of the DNAs were then calculated by using the formula of Schildkraut et al (21).

RESULTS

Colonies formed on NA by the primary forms of the bacteria symbiotic with *Neoaplectana* spp. (groups I through IV) were convex, circular with slightly irregular margins, 1.5 to 2.0 mm in diameter after 4 days, and slightly granular; pigmentation varied with the nematode species (Table 2). The secondary-form colonies of these bacteria were similar to the primary-form colonies but somewhat flatter, wider (diameter, 2.5 to 3.5 mm), and more lightly pigmented. On tergitol-7 medium containing TTC, the primary forms of group I and group II bacteria produced blue and green colonies, respectively, surrounded by decolorized zones after 3 to 5 days. Secondary-form colonies did not absorb bromthymol blue; these colonies were red. Neither form of the group III bacterium absorbed bromthymol blue, and both forms produced red colonies.

On NA, the primary forms of all bacteria symbiotic with *Heterorhabditis* spp. (group V) except isolate T280/1 formed colonies that were mucoid, convex, circular with slightly irregular margins, and 3 mm in diameter after 4 days; isolate T280/1 primary-form colonies were slightly flattened and 4 mm in diameter. The pigmentation of the colonies of some isolates varied with time; all were yellow after 2 days and then some progressed through orange to red or through light brown to pink. The secondary-form colonies of these bacteria were much flatter and wider (4.5 mm after 4 days) than the primary-form colonies, had more irregular margins, and were not mucoid. Two of the secondary-form isolates were further differentiated as follows: isolate T280/2 formed rough colonies and isolate HP/2 formed yellow colonies, whereas isolate T280/1 formed smooth colonies and isolate HP/1 formed orange colonies. Colonies of the primary form on tergitol-7 medium containing TTC were green with orangish brown centers and were surrounded by clearing zones diffused with pigment. None of the secondary-form colonies except those of isolate T280/2 absorbed bromthymol blue from tergitol-7 medium containing TTC.

The results of these tests are summarized in Tables 2 through 5.

DISCUSSION

All of the symbionts of *Neoaplectana* and *Heterorhabditis* examined in this and previous studies should be grouped in the same genus. However, the results of a previous study (1) and

TABLE 3. Characteristics common to all isolates^a

Test	Result
Gram stain	—
Motility	+
Cells peritrichous	+
Oxidase	—
Cytochrome oxidase	—
Peroxidase	—
Hugh and Leifson (open tube)	+
Hugh and Leifson (closed tube)	+
Gelatin liquefaction	+
Casein hydrolysis	+
Deoxyribonuclease	+
KCN	+
Reducing compounds from sucrose	—
Lipase (egg yolk agar)	+
Potato starch hydrolysis	—
Soluble starch hydrolysis	—
Acetoin	—
Methyl red	—
Nitrate reduction	—
Chitinase	—
Protease (Loeffler medium)	—
Protease (egg albumin)	—
Tyrosinase	—
Arginine dihydrolase	—
Lysine decarboxylase	—
Ornithine decarboxylase	—
Glutamic acid decarboxylase	—
TSI (slope, butt, H ₂ S)	Alkaline/acid/—
Utilization of acetate in OY medium	+
Utilization of benzoate	—
Utilization of citrate	+
Utilization of formate	+
Utilization of fumarate	+
Utilization of lactate	+
Utilization of malate	+
Utilization of malonate	—
Utilization of oxalate	—
Utilization of succinate	+
Utilization of tartrate	—
Glucanate utilization	—
(Shaw-Clark medium)	
Growth on MacConkey agar	+

^a In addition, the cells of all isolates were rod shaped.

of this study indicate that the description of the genus *Xenorhabdus* may require revision. The production of two colony forms is characteristic of members of this genus. Although group IV bacteria were available only in one form (probably secondary), subsequent isolations of bacteria from new populations of *Neoaplectana* sp. strain M have yielded a *Xenorhabdus* sp. which occurs in two forms (Akhurst, unpublished data). The dimorphic characteristic should be included in the definition of the genus *Xenorhabdus*. In this study, the bacteria produced acid from some but not all carbohydrates tested, and none of the isolates produced glutamic acid decarboxylase. Some isolates produced phenyl-

TABLE 4. Production of acid from carbon sources by *Xenorhabdus* spp.

Carbon source	Group I	Group II	Group III	Group IV	Group V
Adonitol	—	—	—	—	—
Arabinose	—	—	—	—	—
Cellobiose	3±, 4—	6±, 9—	1±, 1—	3±, 1—	4±, 6—
Dextrin	+w	+w	+w	+w	+w
Esculin	—	—	—	—	—
Fructose	+	14+, 1+w	+	+	+
Galactose	—	—	—	—	—
Glucose	+	+	+	+	+
Glycerol	—	1+w, 9±, 5—	+w	1+w, 3—	—
Inositol	4±, 3—	11±, 4—	1±, 1—	±	8±, 2—
Inulin	—	—	—	—	—
Lactose	—	—	—	—	—
Maltose	+w	+w	1+, 1+w	2+, 2+w	1+, 9+w
Mannitol	—	—	—	—	—
Mannose	+	+	1+, 1+w	+	+
Melezitose	6+w, 1±	13+w, 2±	1+w, 1±	+w	9+w, 1±
Melibiose	—	—	—	—	—
α-Methyl glucoside	—	3±, 12—	—	—	—
β-Methyl glucoside	—	—	—	—	—
Raffinose	—	—	—	—	—
Rhamnose	—	—	—	—	—
Ribose	+w	+w	+w	+w	+w
Saccharose	—	3±, 12—	—	—	1±, 9—
Salicin	—	—	—	3+w, 1—	2±, 8—
Sorbitol	—	3±, 12—	—	—	1±, 9—
Sorbose	—	—	—	—	—
Sucrose	2±, 5—	2±, 13—	1±, 1—	—	1±, 9—
Trehalose	+w	+w	+w	1+, 3+w	9+w, 1±
Xylose	3±, 4—	5±, 10—	—	1±, 3—	1±, 9—

alanine deaminase or urease or both, and all produced lipase on egg yolk agar. Some did not form blue colonies on tergitol-7 medium containing TTC. The G+C contents of the DNAs were outside the range reported by Thomas and Poinar for *Xenorhabdus* (25).

Differences between my results and the results of Thomas and Poinar (25) may be due in part to differences in the methods used. In this study bromcresol purple was used to determine acid production from carbohydrates because the bacteria produced enough acid from the basal

medium to affect bromthymol blue. Phenylalanine deaminase production was tested after 5 and 7 days, as well as after 2 days as prescribed in the *Difco Supplementary Literature* (8) because results obtained after 2 days were inconsistent. Citrate utilization was tested on OY agar rather than on Simmons citrate agar because the isolates grew poorly on the latter. Lipase activity was tested with fresh egg yolk emulsion rather than with egg yolk extract. The methods used in this study to detect urease and the utilization of organic acids may also have been different from

TABLE 5. DNA base ratios of some *Xenorhabdus* isolates

Bacterial isolate	Group	Nematode species	Bouyant density (g/cm ³)	G+C content (mol%)
<i>M. luteus</i> ^a			1.731	72.4
A24	I	<i>N. feltiae</i> (= <i>N. carpocapsae</i>)	1.707	48.0
A25	I	<i>N. feltiae</i> (= <i>N. carpocapsae</i>)	1.707	48.0
T231	II	<i>N. bibionis</i>	1.706	46.9
G/2	III	<i>N. glaseri</i>	1.708	49.0
N37	IV	<i>Neoaplectana</i> sp. strain N	1.709	50.0
T171	IV	<i>Neoaplectana</i> sp. strain M	1.708	49.0
T310	V	<i>Heterorhabditis</i> sp.	1.707	48.0

^a Included as a standard.

TABLE 6. Characteristics distinguishing among the subspecies and group IV of *X. nematophilus*

Taxon	Host nematode	Pigmen- tation	Phospha- tase	Growth at:		Bromthymol blue absorbed by primary form	Lecithinase produced by primary form	Lipase (Tween 80 test) produced by primary form	Phenylalanine deaminase produced by secondary form	Red colonies on MacConkey agar (primary form)
				34°C	40°C					
<i>X. nematophilus</i> subsp. <i>nematophilus</i>	<i>N. felitiae</i> (= <i>N. carpocapsae</i>)	Buff	- ^a	+	-	+	+	-	+	-
<i>X. nematophilus</i> subsp. <i>bovienii</i>	<i>N. bibionis</i>	Yellow	- ^b	-	-	+	+	+	-	-
<i>X. nematophilus</i> subsp. <i>poinarii</i>	<i>N. glaseri</i>	Brown	-	+	+	-	-	+	-	+
Group IV	<i>Neoaplectana</i> sp. M or N	Brown	+	+	-	?	?	?	?	?

^a +, Positive reaction; -, negative reaction; ?, reaction unknown. Only one form (probably secondary) was available.^b One of 14 isolates produced a positive reaction.

those of Thomas and Poinar (25), which were not specified.

There must be some doubt about the range of the G+C contents of the DNAs of *Xenorhabdus* spp. In describing the genus, Thomas and Poinar (25) give values of 43 to 44 mol% for nine isolates, whereas Khan and Brooks (11) recorded a value of 45.6 mol% for one of those isolates and in this study values ranged from 46.9 to 50.0 mol% for another seven isolates. Although these differences in G+C content of DNA may only reflect differences in methods of determination, this part of the genus description obviously requires more attention.

Thomas and Poinar (25) listed only two species in the genus *Xenorhabdus*, *X. nematophilus* (the symbionts of *N. felitiae*, *Neoaplectana bibionis*, and some unidentified *Neoaplectana* spp.) and *X. luminescens* (the symbionts of *Heterorhabditis* spp.). In this study I show that the bacterial symbionts of any one species of *Neoaplectana* are consistently similar and are distinguishable from the bacteria symbiotic with any other species. Thomas and Poinar (25) did not distinguish among the symbionts of their various *Neoaplectana* spp. However, since these authors had only a small number of isolates, separation would not have been justified. The characteristics that distinguish these groups of bacteria (groups I through IV) from each other are few (Table 6) and probably are insufficient to warrant division of the groups into separate species. Moreover, none of the bacteria in groups I through IV showed catalase or peroxidase activity, which is unusual in facultatively anaerobic bacteria. Since the absence of such activity has not been shown to be required in the interaction between nematodes and bacteria it must be considered a good argument for classifying groups I through IV as a single species, *X. nematophilus*. However, the groups should be distinguished taxonomically because, except for group IV, each bacterial group is found only in association with one nematode species, and although the infective stage of a strain of *Neoaplectana* transmits bacteria belonging to the same group as the natural symbiont, only a very small proportion of infective-stage nematodes can transmit bacteria from another group (Akhurst, unpublished data). Since these groups can also be identified by other characteristics (Table 6), they should be considered subspecies. It would be premature to describe a subspecies for group IV since it is very likely that the primary form of these bacteria has not been studied. The proposed subspecies, whose differentiating characteristics are shown in Table 6, are as follows: *Xenorhabdus nematophilus* subsp. *nematophilus* for bacteria symbiotic with *N. felitiae* (type strain, ATCC

19061); *Xenorhabdus nematophilus* subsp. *bovienii* (bo.vi.en'i.i. M.L. gen. n. *bovienii* of Bovien; named for P. Bovien, who first reported the presence of bacteria in the intestinal lumen of the *N. bibionis* infective stage) for bacteria symbiotic with *N. bibionis* (type strain, T228 [= University of Queensland Microbiology Collection UQM 2210]); and *Xenorhabdus nematophilus* subsp. *poinarii* (poi.nar'i.i. M.L. gen. n. *poinarii* of Poinar; named for G. O. Poinar, Jr., who has made major contributions to the understanding of the nematode-bacterial association and was involved in isolating the symbiont of *N. glaseri*) for bacteria symbiotic with *N. glaseri* (type strain, G1 [= UQM 2216]).

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