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Uric Acid-Degrading Bacteria in Guts of Termites [*Reticulitermes flavipes* (Kollar)]†

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Uricolytic bacteria were present in guts of *Reticulitermes flavipes* in populations up to 6 × 10^4 cells per gut. Of 82 strains isolated under strict anaerobic conditions, most were group N *Streptococcus* sp., *Bacteroides termitidis*, and *Citrobacter* sp. All isolates used uric acid (UA) as an energy source anaerobically, but not aerobically, and NH_3 was the major nitrogenous product of uricolysis. However, none of the isolates had an absolute requirement for UA. Utilization of heterocyclic compounds other than UA was limited. Fresh termite gut contents also degraded UA anaerobically, as measured by $^{14}CO_2$ evolution from [2-^{14}C]UA. The magnitude of anaerobic uricolysis [0.67 pmol of UA catabolized/(gut × h)] was entirely consistent with the population density of uricolytic bacteria in situ. Uricolytic gut bacteria may convert UA in situ to products usable by termites for carbon, nitrogen, energy, or all three. This possibility is consistent with the fact that *R. flavipes* termites form UA, but they do not void the purine in excreta despite the lack of uricase in their tissues.

Uric acid (UA) is a major nitrogenous excretory product of many terrestrial insects (6, 7), and it is well suited to this purpose. Because of its poor solubility in water, UA can be defecated as a nontoxic solid. Water loss during excretion is thereby minimized (6, 7). However, UA should not be regarded as merely a nitrogenous waste product of insects. For example, *Periplaneta americana* cockroaches store UA internally and use it as a metabolic reserve when placed on nitrogen-deficient diets (24, 25). UA nitrogen mobilized under such conditions is used in part for oothecal production by females (25). Utilization of UA apparently occurs in some other insects as well (7).

Intuitively, one might expect that xylophagous termites, which thrive on nitrogen-poor diets, would have evolved efficient means of conserving combined nitrogen. One strategy to accomplish this was envisioned by Leach and Granovsky (19). These workers hypothesized that UA, elaborated into the termite gut via the Malpighian tubules, is degraded by the hindgut microbiota to a form of nitrogen reusable by the insects. A corollary to their hypothesis is that carbon atoms of UA are also reused. Leach and Granovsky performed no experiments to test their provocative notion, although the abundance of microbes in the termite hindgut (4, 5) makes their suggestion plausible.

A major interest in our laboratory is the role of gut organisms in termite nutrition. We therefore sought to critically test the UA-recycling hypothesis. In a previous paper (28), we reported that termites form UA, but they do not void the purine despite the lack of uricase in termite tissues. We now present data to reconcile this apparent anomaly and which support Leach and Granovsky's hypothesis. Herein we document the presence of uricolytic bacteria in guts of *Reticulitermes flavipes* termites. The present paper deals with the isolation, identification, and nutrition of the bacteria, as well as estimation of their population levels in situ. An accompanying paper (29) concerns the anaerobic metabolism of UA by pure cultures.

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MATERIALS AND METHODS

Insects. *R. flavipes* (Kollar) termites were collected in Janesville, Wis., Dansville, Mich., and Spring Arbor, Mich., and were maintained in the laboratory as previously described (31). UA content of termites was determined by enzymatic assay after extraction from tissue with Li_2CO_3 (28).

P. americana L. cockroaches were obtained from the Pesticide Research Center of Michigan State University. They were maintained on a commercial dog food diet and were fed water ad lib.

Media and culture techniques. Strict anaerobic techniques were used for preparation of liquid and solid media (15, 17, 31) unless indicated otherwise.

The composition of culture media is reported as percentage (wt/vol), unless indicated otherwise. Double-layer agar plates were used to isolate uricolytic bacteria and contained 0.1 and 1.0% UA in the bottom and top layers, respectively (1). Isolation media were designated SUA, BHIU, and TYU. SUA was the supplemented uric acid agar of Barnes and Impey (1) with

the following exceptions: 0.09% beef extract (Difco Laboratories, Detroit, Mich.) plus 0.15% peptone (Difco) replaced Lab-Lemco, and 0.1% liver concentrate (Wilson Diagnostics) replaced liver extract. BHIU medium was supplemented brain-heart infusion (BHI) medium (15) containing UA as indicated above. TYU agar medium contained: tryptone (Difco), 1.0%; yeast extract (Difco), 0.1%; salts solution (15) minus $NaHCO_3$, 4% (vol/vol); cysteine·HCl, 0.05%; resazurin, 10^{-4}%; and UA as indicated above. All solid media contained 1.5% agar.

TYU broth medium consisted of TY basal medium into which NaOH-solubilized UA was incorporated. TY basal was similar to TYU agar (above), but lacked UA, agar, resazurin, and cysteine·HCl. For addition to sterile TY broth, UA was solubilized as a 2% solution in 0.5 N NaOH, filter sterilized (filter type GS; 0.22-µm pore size; Millipore Corp., Bedford, Mass.), and added at a final concentration of 0.1%. A predetermined amount of sterile 0.5 N HCl was then added to neutralize the medium before inoculation. TYFU broth medium was identical to TYU, but also contained filter-sterilized fructose at a final concentration of 0.04%. In general, TY-based broth media were prepared in air by heat sterilization and rapid cooling of the TY basal portion, followed soon thereafter by additions as described above and then replacement of the head space atmosphere with O_2-free gas. However, for culture of bacteroides the TY basal portion was prereduced and anaerobically sterilized (15). Culture vessels were then transferred into a vinyl anaerobic glove box (Coy Manufacturing Co., Ann Arbor, Mich.) containing an O_2-free atmosphere of N_2/H_2 (90/10, vol/vol) wherein appropriate additions to the medium were made. The head space atmosphere of culture vessels was replaced with N_2/CO_2 (95/5, vol/vol) after inoculation.

Semi-D basal medium, used to conduct fermentation balances with growing cells, contained: Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 1.0%; salts solution (15) minus $NaHCO_3$, 4% (vol/vol); $MnCl_2 \cdot 4H_2O$, 0.005%; adenine, thymine, guanine, cytosine, and uracil, 5×10^{-4}% each; riboflavin, calcium pantothenate, nicotinic acid, pyridoxine·HCl, and folic acid, 10^{-4}% each; thiamine-HCl, 10^{-5}%; and biotin, 10^{-7}%. A chemically defined medium, D medium, was identical to semi-D medium but lacked Trypticase.

Small volumes (e.g., 10 ml) of broth media were usually contained in 18-mm anaerobe tubes (Bellco Glass, Inc., Vineland, N.J.) equipped with rubber stoppers. Mass culture of cells was done by using aspirator bottles of various sizes and employing O_2-free N_2 in the head space. Aerobic broth culture was as previously described (27).

Isolation of uricolytic bacteria. Removal of termite guts and isolation of bacteria was done as described previously (27, 31), except that gut homogenates were prepared in TY broth and treated in a microblender (Eberbach) for 60 s before dilution and plating. Isolation plates were incubated in an anaerobic glove box and were supplemented with CO_2 (31). After 7 days, presumptive uricolytic isolates were randomly picked from among those colonies surrounded by a clear zone in the otherwise opaque medium (1). Such colonies were easily recognizable, even against a background of 100-fold excess nonuricolytic colonies. Isolates were considered to be pure cultures after three successive passages on streak plates; microscopic observations, including Gram stains of cells, verified this conclusion.

Control experiments used the following inocula: surface washes of intact termites; and homogenates prepared from degutted termite bodies.

Characterization of isolates. General characterization of isolates was done as previously described (27, 31). Acidic fermentation products were determined after anaerobic growth of isolates in BHI containing 0.5% glucose (BHIG). Media for tests with *Bacteroides* isolates were prepared as described by Dowell and Hawkins (11). Spore formation was routinely evaluated by observing wet mounts or malachite green-stained preparations of cells by phase-contrast or bright-field microscopy, respectively. For *Bacteroides* isolates, spore formation was assessed while cells were growing on chopped meat agar slants for 3 weeks (15). In addition, the heat resistance of *Bacteroides* in starch broth was tested (15).

The moles percent guanine plus cytosine (G+C) content of deoxyribonucleic acid (DNA) of *Bacteroides termitidis* was determined by buoyant density analysis. Cells were grown in TY broth supplemented with 1% glucose, 0.024% DL-threonine, and [*methyl*-^{3}H]thymidine (10 µCi/ml) to radioactively label the DNA. DNA was partially purified by the method of Marmur (21), except that only two deproteinization treatments were used, and these were followed by a single precipitation step with isopropanol. The buoyant density of such preparations in CsCl gradients was determined as described by Schildkraut et al. (30). Samples were centrifuged at 20°C in a Beckman model 50Ti rotor at 40,000 rpm for 60 h. Centrifuge tubes were then punctured from the bottom, and fractions (10 drops each) were collected. Fifty microliters of each fraction was applied to trichloroacetic acid-impregnated filter paper squares, which were then washed, dried, and analyzed for radioactivity (12). The refractive index of fractions containing peak radioactivity was measured by using an Abbe 3-L refractometer (Bausch & Lomb, Inc., Rochester, N.Y.)., and from this the moles percent G+C in DNA was calculated (30). DNA from *Escherichia coli* B (ρ = 1.710; 30), prepared as described above but labeled by growth of cells with [*methyl*-^{14}C]thymidine (0.1 µCi/ml culture), was used as an internal standard.

Nutrition and growth studies. Most nutrition studies were done with the growth-limiting TY basal medium (above) to which additions were made. Heterocyclic compounds to be added were solubilized as described above for UA. Exceptions were inosine, purine, and caffeine (which were prepared as 2% stock solutions in water), and xanthine (which was prepared as a 1% stock solution in 0.5 N NaOH). All test substrates were filter sterilized (see above). Where necessary, pH adjustment was made with HCl (above) before inoculation. Some nutritional studies were done with medium D.

Growth of cells was measured turbidimetrically at 660 nm by using a Spectronic 20 colorimeter (Bausch & Lomb). Absorbance readings were converted to cell numbers by means of standard curves relating the

reading to direct microscopic counts (*Streptococcus*) or viable cell counts (*Bacteroides* and *Citrobacter*). Unless otherwise stated, cultures were incubated at 30°C (*Streptococcus*) or 37°C (*Bacteroides* and *Citrobacter*).

Assay for UA, NH_3, and urea in spent growth media was done after removal of cells by centrifugation at 12,000 × *g* for 10 min. UA was assayed spectrophotometrically at 292 nm by using hog liver uricase (uric acid diagnostic kit no. 292-UV; Sigma Chemical Co., St. Louis, Mo.). Urea was assayed by determining urease-dependent NH_3 production with phenol nitroprusside reagent (urea nitrogen diagnostic kit no. 640; Sigma). NH_3 was determined by using the urea assay reagents without urease.

Anaerobic metabolism of UA by minced termite guts. Guts were removed as described above from 100 *R. flavipes* workers and pooled into a small watch glass containing 500 μl of 0.1 M potassium phosphate buffer (pH 7.0). Guts were then minced with a scalpel, and transferred to a 13-mm test tube, and the suspension was made up to 1,500 μl with buffer. The suspension was then subject to agitation in a Vortex blender at high speed for 20 s. Five hundred microliters of such preparations was used per reaction mixture. Individual reaction mixtures (620 μl) contained: potassium phosphate buffer (pH 7.0), 52 μmol; dithiothreitol, 1 μmol; [2-^{14}C]UA, 2 to 3 nmol; sodium formate (optional), 1 μmol; and 30 to 40 minced gut equivalents.

Reactions were performed under 90% N_2/10% H_2 in 5-ml serum vials fitted with stoppers. Suspended from each stopper was a center well assembly (Kontes, Vineland, N. J.; catalog no. K-882320). Reactions were initiated by addition of UA and were terminated after 3 h by injection of 150 μl of 5 N HCl. Phenethylamine (200 μl) was then injected into the center well and allowed to absorb $^{14}CO_2$ for one additional hour, after which time radioactivity was estimated.

Radioactivity measurements. Radioactivity measurements and quench corrections were made as described previously (32).

Microscopy of insect fat body tissue. Fat body tissue was dissected into a primary fixative of 2% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.4) and then held at 4°C for 2 h. After primary fixation, tissue was washed three times in glutaraldehyde-free phosphate buffer and postfixed in phosphate buffer containing 1% OsO_4. Specimens were then dehydrated with ethanol, cleared with propylene oxide, and embedded in Epon (20). Sections (1 to 3 μm thick) were cut with a diamond knife and were viewed directly by phase-contrast microscopy or by bright-field microscopy after staining with buffered azure II (18).

Other bacterial strains. For comparative purposes and as positive or negative controls, various known bacterial strains were used. Termite gut heterotrophic bacteria previously isolated on nonselective medium (31) were part of this laboratory's culture collection. *Proteus mirabilis, Citrobacter freundii, Staphylococcus aureus,* and *E. coli* B were obtained from the culture collection of the Department of Microbiology and Public Health, Michigan State University. *Streptococcus lactis* ATCC 19435, *S. cremoris* ATCC 19257, *S. faecalis* ATCC 19433, and *S. faecium* ATCC 19434 were obtained from the American Type Culture Collection, Rockville, Md. *S. lactis* C2 was obtained from L. L. McKay, Department of Food Science and Nutrition, University of Minnesota, St. Paul.

Chemicals. Heterocyclic substrates were obtained from Sigma Chemical Co. and were of the highest purity available. [2-^{14}C]UA was obtained from Amersham Corp., Arlington Heights, Ill. All other chemicals were of reagent grade and were obtained from various commercial sources.

RESULTS

Isolation and identification of uricolytic bacteria. Over a period of 3 years, uricolytic bacteria were consistently isolated from guts of *R. flavipes*, regardless of the termites' origin or length of captivity (Table 1). Population levels ranged from $<10^2$ to 6.1×10^4 cells/gut, with a grand mean of $3.5 \pm 1.7 \times 10^4$ (excluding the data from experiments 7 and 8). We do not know why so few uricolytic bacteria were detected in experiments 7 and 8 (Table 1). Control experiments (Table 1, footnote *d*) verified that uricolytic isolates were of gut origin. Light microscopy failed to reveal bacteria within termite fat body tissue cells. However, typical mycetocytes (i.e., specialized host tissue cells containing endosymbiotic bacteria) (8) were readily observed in fat body tissue of *P. americana* cockroaches used as a control.

The UA content of termites ranged from 0.4 to 31.1% of the insects' dry body weight (Table 1). As documented previously (28), UA stores of *R. flavipes* generally increase during laboratory captivity, yet no clear correlation existed between the UA content of termites and the number of uricolytic gut bacteria.

Of 117 strains initially isolated, 35 lost the uricolytic phenotype upon subculture from primary isolation plates, and they were not examined further. Of the 82 strains that retained the phenotype as a fairly stable characteristic, most were identified as group N *Streptococcus* sp., *B. termitidis*, and *Citrobacter* sp. One strain of group D *Streptococcus* was obtained.

General physiological and biochemical characteristics of the group N streptococcal isolates are presented in Table 2. Lactate was the major acidic fermentation product (Table 2), suggesting that cells were homolactic. Detailed studies with one isolate (strain UAD-1) verified this notion. When growing anaerobically in semi-D medium containing excess (i.e., 1%) glucose, strain UAD-1 formed (millimoles/100 mmol of glucose fermented): lactate, 162.5; acetate, 21.1; ethanol, 5.2; formate, 5.0; and CO_2, 7.0. Similarly, products from 1% fructose were (millimoles/100 mmol of fructose fermented): lactate, 168.4; ace-

TABLE 1. *Uricolytic bacteria present in guts of R. flavipes*

Expt no.	Termites[a]				No. of uricolytic bacteria/gut[d]	No. of isolates examined	Identification of isolates			
	Origin[b]	Date collected	Length of captivity (mo)[c]	UA content (% of dry wt)			Group N *Streptococcus*	Group D *Streptococcus*	*Citrobacter* sp.	*B. termitidis*
1	J	June 1976	1	ND[e]	3.8×10^4	20	18	0	2	0
2			4	ND	3.9×10^4	23	13	1	9	0
3			10	ND	2.8×10^4	15[f]				
4	J	June 1977	0	1.7	1.8×10^4	20[f]				
5			4	15.1	4.5×10^4	8	0	0	0	8
6			5	24.6	1.5×10^4	3	0	0	0	3
7			10.5	31.1	$<10^2$	0				
8	J	June 1978	0	2.0	$<10^2$	0				
9			8	18.0	3.2×10^4	10	0	0	10	0
10	J	June 1979	0	3.3	0.6×10^4	4	1	0	0	3
11	D	June 1979	0	4.0	5.6×10^4	6	6	0	0	0
12			2	0.4	6.1×10^4	3	3	0	0	0
13	D	August 1979	0	1.7	4.9×10^4	5	5	0	0	0

[a] All termites were workers, except for experiment 6 (brachypterous larvae).

[b] J, Janesville, Wis.; D, Dansville, Mich.

[c] Zero months refers to termites used within 24 h of collection.

[d] Mean value of two to six replicates made with SUA plates. No uricolytic bacteria were detected in surface washes of intact termites or in homogenates of degutted termite bodies.

[e] ND, Not determined.

[f] Isolates lost uricolytic ability upon subculture and were not studied further.

tate, 22.9; ethanol, 13.8; formate, 16.6; and CO_2, 9.1. However, the overall properties of group N isolates did not correspond exactly to those of a known species (9, 13). Uricolytic strains failed to produce NH_3 from arginine (distinguishing them from *S. lactis*), yet showed growth at 40°C, at pH 9.2, and in the presence of 0.3% methylene blue (distinguishing them from *S. cremoris*) (Table 2). Preliminary examination of lactate dehydrogenase activity of strain UAD-1 suggested similarities to that of *S. faecium*, a group D *Streptococcus* (13; E. Garvie, personal communication). The mol% G+C in the DNA of strain UAD-1 was 36.6 (E. Garvie, personal communication), a value in the middle of the range found for the genus *Streptococcus*. Based on these results, we deferred species assignment of the group N isolates.

B. termitidis isolates were acidogenic rods 0.5 × 3.1 μm in size. Their properties corresponded closely to those of known *B. termitidis* (14, 33), although the present isolates did not produce formate as a fermentation product and formed small amounts of acid from lactose in a delayed (5 days) reaction. In addition, all strains produced acid from mannitol, mannose, rhamnose, and trehalose, but not from melezitose. The mol% G+C in the DNA was 35.6 (strain UAD-50).

Citrobacter isolates were indole positive, similar to strains previously isolated on nonselective media (31). These were not assigned to a species.

Nutritional studies. Nutritional studies on the isolates were done to confirm UA utilization; to examine the role of UA as a carbon, nitrogen, and energy source for growth; and to evaluate their nutritional diversity with respect to heterocyclic compounds in general. UA increased the anaerobic cell yield of all isolates when the purine was incorporated into an otherwise growth-limiting medium (i.e., TY medium). These data suggested that energy was derived from anaerobic uricolysis by the isolates. Results with three representative strains are presented in Table 3. Preliminary experiments with *Streptococcus* UAD-1 revealed that small amounts (0.04%) of fructose markedly stimulated UA consumption and cell yields. Consequently, this sugar was frequently added to TY medium for evaluating the utilization of heterocyclic compounds by all strains. Interestingly, diauxic growth of strain UAD-1 (i.e., a primary logarithmic growth phase separated from a small secondary growth phase by 10 to 20 h) resulted when 0.04% glucose was included with UA. Under these conditions UA was consumed only slightly (i.e., 10% of initial) and only during the secondary phase, after all the glucose was depleted. Diauxic growth was not observed when fructose was included with UA.

Anaerobic cell yields of streptococci and bacteroides were at least twice as great with UA as with identical media lacking this purine (Table 3). In addition, NH_3 production was 79 to 100%

TABLE 2. *Characteristics of uricolytic group N Streptococcus isolates from R. flavipes*

Test or substrate	Reaction[a]
Gram reaction	Variable
Morphology	Ovoid; 0.6 to 0.7 μm in diam
Motility	−
Acidic fermentation products	Lf (as)
Final pH (BHIG medium)	4.5
Relation to O_2	Facultative
Catalase	−
Nitrate to nitrite	−
Growth in:	
BHI + 4.0% NaCl	+
BHI + 6.5% NaCl	−
0.1% methylene blue milk	+
0.3% methylene blue milk	+
Growth in BHI at:	
10°C	+
40°C	+
45°C	−(+)[b]
pH 9.2	+
pH 9.6	+
Heat tolerance[c]	−
Hydrolysis of:	
Esculin	+
Hippurate	−
Arginine	−
Hemolysis	α
Precipitin reaction with group N antiserum	+[d]

[a] Symbols: +, Positive reaction; −, negative. Acidic fermentation products: L, lactic; f, formic; a, acetic; s, succinic. Upper-case and lower-case letters refer to major and minor amounts, respectively (15). Parentheses indicate results with some strains.

[b] Two of 46 strains showed growth at 45°C.

[c] 60°C, 30 min.

[d] Only 12 strains tested.

of theoretical (based on UA consumed).

Although *Citrobacter* isolates readily cleared UA-containing plates, their cell yields were not greatly stimulated by UA in broth media (Table 3). Under the latter conditions, only small amounts of UA were consumed by *Citrobacter* strains, yet such UA consumption was accompanied by NH_3 production. Interestingly, fructose suppressed UA utilization and NH_3 production by all *Citrobacter* strains.

UA was not used as an energy source aerobically by streptococci or citrobacters. This conclusion was based on growth yield determinations as well as enzymatic assay of UA consumption.

The ability of isolates to use other heterocyclic compounds anaerobically was fairly limited (Table 3). *Streptococcus* UAD-1 appeared to catabolize allantoin and allantoic acid only slightly, as indicated by a small increase in cell yield and NH_3 production (Table 3), as well as formation of small amounts of urea (Table 3, footnote *d*). Increased growth of this strain on inosine and on fructose plus guanosine apparently resulted from utilization of the ribose portion of the molecules only, since: (i) the final pH of the media (pH 5.0 to 5.1) was more acidic than that of UA-containing media (pH 7.2 to 7.3); (ii) little or no NH_3 or urea was formed; (iii) the purine portion of the ribosides accumulated in (and in the case of guanosine alone, crystallized out of) the medium and was readily identified by thin-layer chromatography; and (iv) hypoxanthine and guanine alone were not used although ribose was. No other purines or pyrimidines tested were used by strain UAD-1.

B. termitidis UAD-50 appeared to use inosine and hypoxanthine; utilization of xanthosine was questionable. Interestingly, xanthine and guanosine inhibited growth of strain UAD-50. *Citrobacter* UAD-25 showed slightly more nutritional versatility than either strain UAD-1 or UAD-50. Utilization of guanosine, hypoxanthine, xanthine, and xanthosine was obvious from the increase in NH_3 production. Inosine utilization was questionable: although cell yields were increased, there was no increase in NH_3 production.

By using a chemically defined medium (medium D), we found that none of the representative strains used UA as sole carbon, nitrogen, and energy source for anaerobic growth. *Streptococcus* UAD-1 would not use 0.1% UA as sole nitrogen or sole energy source with 0.4% fructose or 1.0% Trypticase, respectively, but would use UA as an additional energy source with 0.04% fructose plus 1.0% Trypticase. Two *B. termitidis* strains tested behaved identically to *Streptococcus* UAD-1, except that one strain (UAD-55) used UA as sole nitrogen source in the presence of 0.4% fructose.

Comparative tests with other bacterial strains. Reference strains of group N streptococci (i.e., *S. lactis* ATCC 19435 and *S. cremoris* ATCC 19257) did not use UA when growing anaerobically in TYU or TYFU broth media, nor did these strains form clear zones on TYU agar plates. Tests were also done on *S. lactis* C2, as well as 54 group N streptococci previously isolated from guts of *R. flavipes* (31) and which included *S. lactis*, *S. cremoris*, and strains of unknown species. None used UA anaerobically as judged by lack of clear zone formation on UA-containing plates. Of *Citrobacter* strains isolated previously (31), only indole-positive biotypes cleared UA-containing plates. Previously isolated *Bacteroides* strains (31) were not tested for uricolytic ability, although the characteris-

TABLE 3. *Anaerobic utilization of uric acid and other heterocyclic compounds by growing cells*[a]

Added[b] to TY basal medium	*Streptococcus* UAD-1		*B. termitidis* UAD-50		*Citrobacter* UAD-25	
	Yield[c] (cells/ml × 10^8)	NH_3 produced[d] (μmol/ml)	Yield (cells/ml × 10^8)	NH_3 produced (μmol/ml)	Yield (cells/ml × 10^8)	NH_3 produced (μmol/ml)
No addition	2.0	0.0	0.4	1.2	1.3	7.3
Uric acid	4.2	7.3 (2.3)	1.4	17.3 (4.9)	1.4	12.8 (1.4)
F	5.8	0.2	1.3	0.7	1.9	4.5
F + uric acid	11.5	23.5 (6.1)	2.6	23.3 (6.1)	2.4	5.1 (0.3)
Allantoin	2.3	2.3	0.4	1.3	1.1	7.9
F + allantoin	6.4	2.5	1.4	0.9	ND[e]	ND
Allantoic acid	1.8	0.4	0.4	1.0	1.0	8.3
F + allantoic acid	6.6	3.6	1.4	0.8	ND	ND
Inosine	5.8	0.3	2.1	7.8	3.0	7.0
F + inosine	6.3	0.3	2.4	5.8	ND	ND
Guanosine	—[f]	0.2	0	ND	3.3	18.7
F + guanosine	7.1	0.3	0	ND	ND	ND
Hypoxanthine	1.9	0.0	2.3	8.9	3.0	16.2
F + hypoxanthine	6.1	0.2	2.7	9.1	ND	ND
Xanthine	1.3	0.3	0	ND	1.6	15.7
F + xanthine	4.7	0.4	0	ND	ND	ND
Xanthosine	1.5	0.0	0.5	ND	2.5	25.3
F + xanthosine	5.5	0.0	1.3	4.3	ND	ND
Ribose	3.3	0.0	0.9	1.2	1.3	6.0
F + ribose	7.5	0.0	ND	ND	ND	ND

[a] The following compounds were not used by growing cells: adenine, guanine, purine, caffeine, thymine, cytosine, uracil, and orotic acid.

[b] Final concentration of all heterocyclic compounds was 0.1% except for guanine (0.05%). Fructose (F) and ribose were used at a concentration of 0.04 and 0.05%, respectively. Incubation was under N_2/CO_2 (95/5, vol/vol). Initial pH of media was 7.0 ± 0.2.

[c] Maximum yields achieved between 24 and 48 h (UAD-1) or 36 and 96 h (UAD-50 and UAD-25). A yield of 0 designates <5 × 10^6 cells/ml.

[d] Trace amounts (0.05 to 0.7 μmol/ml) of urea were formed by strain UAD-1 from allantoin and allantoic acid only. Urea was not formed from uric acid by strains UAD-50 and UAD-25. Decomposition of any of the heterocyclics to yield NH_3 or urea did not occur in uninoculated media. Values in parentheses indicate micromoles of uric acid consumed per milliliter during growth; initial concentration of uric acid was 6.1 μmol/ml.

[e] ND, Not determined.

[f] Cell yields were not determined because guanine crystals formed in the medium.

tics of such strains differed significantly from those of *B. termitidis* isolates obtained in the present study.

Anaerobic metabolism of UA by minced termite guts. We attempted to detect anaerobic metabolism of UA in fresh termite gut contents. Since 60% or more of UA carbon is evolved as CO_2 by isolates (29), $^{14}CO_2$ evolution from [2-^{14}C]UA was used as an estimate of uricolysis by minced guts. Results (Table 4) showed that such preparations degraded a significant amount of UA under strict anaerobic conditions. Little or no stimulation of uricolysis was achieved by including formate in reaction mixtures.

The magnitude of uricolysis by minced guts was consistent with the density of uricolytic bacteria in guts (see Discussion).

DISCUSSION

Results presented herein show that uricolytic bacteria are present in guts of *R. flavipes*, generally in populations of 3.5 ± 1.7 × 10^4 cells/gut. This density is roughly 10% that of total cultivable heterotrophs (31). Nevertheless, we believe such populations to be numerically significant, based on arguments developed previously (31) regarding the tiny size of *R. flavipes* guts. Results are consistent with Leach and Granovsky's hypothesis (19), and also with our previous findings (28) that *R. flavipes* termites form UA but do not void the purine despite their lack of uricase. Presumably UA is catabolized by bacteria when it enters the gut, and therefore it is not found in the feces. However, the dynamics of UA mobilization and bacterial uricolysis in situ, as well as the nutritional control of these processes, are important points which remain to be defined.

Uricolytic isolates were most likely derived from the hindgut, although a small portion of midgut usually remains attached to extracted hindguts (5, 31). Previous studies (31) showed that populations of heterotrophs obtained from midgut homogenates were only 1/10 that of the present uricolytic isolates. However, Malpighian tubules of *R. flavipes* empty at the precise junction of mid- and hindgut, i.e., at the enteric valve (26). Thus, some uricolytic bacteria could conceivably colonize the midgut, near the enteric valve, yet still have ready access to UA.

TABLE 4. *Anaerobic metabolism of [2-^{14}C]UA by minced guts of R. flavipes workers*[a]

Added[b] to reaction mixture (nmol)	$^{14}CO_2$ evolved		pmol of substrate ^{14}C evolved as $^{14}CO_2$/gut equivalent
	dpm	pmol	
[2-^{14}C]UA (2.5)	7,601	67.8	2.06
[2-^{14}C]UA (2.5) + sodium formate (1,000)	7,707	68.7	2.08
Control	0	0	0

[a] Termites collected in Spring Arbor, Mich., and held captive for 7 months before assay.

[b] Specific activity of [2-^{14}C]UA was 50 nCi/nmol. Individual reaction mixtures contained 33 gut equivalents. Incubation was at 25°C for 3 h. Control mixture (lacking formate) was terminated with HCl immediately after UA addition.

Three major groups of uricolytic bacteria were associated with *R. flavipes:* group N *Streptococcus* sp., *B. termitidis*, and *Citrobacter* sp. Their generic affiliation was the same as that of major heterotrophs isolated previously on nonselective medium (31). Barnes and Impey (1) found a greater phenotypic diversity of uricolytic bacteria isolated from chicken ceca, although streptococci and bacteroides were among those isolated. Fecal streptococci of human and sheep origin have also been shown to degrade UA (23). Our isolation of uricolytic *B. termitidis* parallels that of Sebald (33), who isolated this species (referred to by her as *Sphaerophorus siccus* var. *termitidis*) from gut contents of *Reticulitermes lucifugus* (14). By contrast, Donnellan and Kilby (10) reported the isolation of an aerobic, uricolytic vibroid bacterium from fat body tissue of American cockroaches. Uricase mediated the first step of uricolysis by the vibroid (10). As reported herein, we have never observed bacteria of any type in fat body tissue of *R. flavipes*. Moreover, ultrasensitive assays for uricase failed to reveal such activity in fat body tissue (28).

Estimates of anaerobic UA metabolism by minced gut preparations suggested an activity of about 2 pmol of UA catabolized/(gut equivalent × 3 h) (Table 4), which is equivalent to 0.67 pmol/(gut × h). Considering that 10^{11} cells of *Streptococcus* UAD-1 or *B. termitidis* UAD-50 can degrade approximately 20 μmol of UA during a 2-h incubation (see Fig. 2 and 3 and Table 2, 3, and 5 of reference 29), it can be calculated that the in situ density of such bacteria would have to be about 0.67×10^4 cells/gut to account for the activity of minced gut preparations. The latter value compares extremely well with results of enumeration studies (Table 1), indicating that the uricolytic activity of fresh gut contents can be completely accounted for by isolates obtained in pure culture.

Uricolytic isolates displayed limited nutritional versatility with respect to heterocyclic compounds other than UA (Table 3). This was especially true for *Streptococcus* UAD-1. Such metabolic specialization of the isolates undoubtedly relates to the ability of their termite host to form UA (28). The ultimate benefit of uricolytic bacteria to *R. flavipes*, however, can only be inferred at the present time. Acetate, a major carbonaceous product of uricolysis by the isolates (29), is also a major product of cellulolysis in the termite gut (4) and it is a source of carbon and energy for the insect (2, 4, 22). On the other hand, the status of NH_3, the major nitrogenous product of uricolysis, is less clear. Ammonia nitrogen could cycle back to the insects directly, or indirectly after assimilation into microbial protoplasm or other microbial metabolites as suggested by Leach and Granovsky (19). Interestingly, such a cycle appears to occur between the UA-forming marine flatworm *Convoluta roscoffensis* (which also lacks uricase) and its intracellular, uricolytic algal symbiont *Platymonas convolutae* (3, 16). In this case, NH_3 formed during uricolysis by *P. convolutae* is apparently used by the alga for synthesis of amino acids, some of which are exported to the flatworm host. Whatever may be the exact flow of UA nitrogen in *R. flavipes*, it seems clear that uricolysis by gut bacteria could be extremely important to nitrogen conservation within the termite colony.

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