

Degradation of Uric Acid by Certain Aerobic Bacteria

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We have isolated and identified nine cultures of aerobic bacteria capable of growing on an elective medium containing uric acid as the only source of carbon, nitrogen, and energy. Four of these cultures were identified as *Aerobacter aerogenes*, two as *Klebsiella pneumoniae*, and the remainder as *Serratia kiliensis*, *Pseudomonas aeruginosa*, and *Bacillus* species. Another culture identified as *P. fluorescens* required both glucose and uric acid for growth. When 23 laboratory stock cultures were inoculated into the uric acid medium, *A. aerogenes*, *B. subtilis*, *Mycobacterium phlei*, *P. aeruginosa*, and *S. marcescens* were able to grow. These five cultures also grew when the uric acid was replaced with adenine, guanine, hypoxanthine, xanthine, or allantoin, but growth was poor. In all of these media, including the uric acid medium, addition of glucose along with the nitrogenous compounds yielded good growth. Induction experiments demonstrated that the ability of *A. aerogenes*, *K. pneumoniae*, *P. aeruginosa*, *P. fluorescens*, *S. kiliensis*, *S. marcescens*, *B. subtilis*, and *Bacillus* sp. to degrade uric acid is an induced property. Of these organisms, only *Bacillus* sp. accumulated a small amount of intracellular uric acid.

Over-production and accumulation of uric acid, especially in joints, in humans and also in birds, results in a painful metabolic abnormality known as gout. It is known that when uric acid is administered to human subjects by intravenous injections, about 80% of the uric acid is excreted in the urine. If administered by mouth, uric acid is extensively degraded in the gastrointestinal tract, presumably by bacterial action (6, 11). However, not much is known about the degradation of uric acid by various bacteria. Among anaerobes, *Clostridium* species, extensively studied by Barker and co-workers, decompose uric acid, and the utilization of uric acid for growth is dependent upon an initial reduction of uric acid to xanthine (2, 8). Of the aerobic bacteria, *Pseudomonas* species have been reported to break down uric acid (1, 4). *Bergey's Manual* indicates other aerobic bacteria that may utilize uric acid; however, to our knowledge, no detailed study has been made on any of these species.

In yeast, uptake of uric acid from the surrounding medium, and subsequent increase in uricase activity in cellular extracts and breakdown of uric acid have been noted (9, 10).

In the present investigation, therefore, an attempt was made to isolate intestinal and other aerobic bacteria on an elective medium containing

uric acid as the source of nitrogen. Altogether, 10 cultures of aerobic bacteria capable of growing on such a medium were isolated and identified. Of these, nine cultures were capable of growth with uric acid as the only source of carbon, nitrogen, and energy. These cultures were studied to see whether uric acid is broken down by induced or constitutive enzymes. Also, some laboratory stock cultures were tested for their ability to utilize uric acid as carbon, nitrogen, and energy sources.

MATERIALS AND METHODS

Bacteria and media. Waste materials from chickens and frogs, and sewage samples from the local sewage plant, were inoculated into a broth medium containing 0.02% $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.0005% $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.002% $\text{MnSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.005% CaCl_2 , 1% glucose, 0.25% K_2HPO_4 , 0.5% KH_2PO_4 , and 0.15% uric acid, which was dissolved in 0.075 N NaOH and neutralized with 10% KH_2PO_4 . In certain experiments, 0.1% $(\text{NH}_4)_2\text{SO}_4$ was substituted for uric acid as the nitrogen source. Purification of cultures was made by repeated streaking of cultures into the above medium containing 1.5% agar and either uric acid or $(\text{NH}_4)_2\text{SO}_4$. Isolated cultures were maintained in the medium containing glucose and $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. The pH of the media in all cases was adjusted to 7.2. In some experiments, glucose was

omitted and uric acid was used as the sole source of carbon, nitrogen, and energy. Other purines, when used, were also used in a concentration of 0.15% in 0.075 N NaOH, wherever needed, and neutralized with 10% KH_2PO_4 .

Twenty-three laboratory stock cultures were also inoculated into the uric acid medium and incubated under appropriate conditions to check their ability to utilize uric acid. These cultures were maintained in Nutrient Agar or Brain Heart Infusion (BHI) Agar, or in media recommended by the *Manual of Microbiological Methods* (3).

A Klett-Summerson colorimeter with a blue filter (400 to 465 nm) was employed for measuring growth in terms of turbidity. In reporting results, Klett units were converted to micrograms (dry weight) of cells per milliliter.

Identification of organisms. Routine testing methods for the identification of bacteria described in the *Manual of Microbiological Methods* were followed (3). Identification of organisms was made according to *Bergey's Manual*.

Induction of adaptive enzyme. An initial 10 ml of the $(\text{NH}_4)_2\text{SO}_4$ medium was inoculated with a culture and was allowed to grow overnight. The next day, the entire 10-ml culture was placed in a 1-liter flask containing 500 ml of the medium. The flask was incubated overnight in a shaking incubator at 30 C (25 C for *P. fluorescens*). The cells were harvested at the log phase by centrifugation and were washed twice in distilled water to remove as much residual nitrogen as possible. The washed cells were used for the induction of the enzyme. A 91.5-ml amount of the medium containing only inorganic salts, 2% glucose, and 2.5 g (wet weight) of bacterial cells was placed in a 250-ml flask, and the flask was kept in a shaking incubator for 2 hr to deplete residual nitrogen. At the end of this time, 7.5 ml of uric acid (1.5 mg/ml) dissolved in 0.075 N NaOH and 1 ml of 10% KH_2PO_4 was added to the

flask, and the cells were further incubated for periods ranging from 8 hr to overnight; during this incubation, samples were removed at zero-time and at 30-min intervals to check the disappearance of uric acid. Identical experiments with various cultures, without uric acid but with other chemicals, showed no absorption at 293 nm during comparable intervals of time. The samples removed at various times were centrifuged, the filtrate was diluted 1:10 with distilled water, and the optical density of the filtrate was measured at 293 nm (5). For intracellular accumulation of uric acid, the cells were boiled for 10 min and then centrifuged at high speed; uric acid content of the supernatant fluid was determined at 293 nm (5).

All the spectrophotometric readings were taken with either a Beckman DB-G spectrophotometer or a Beckman DU spectrophotometer.

RESULTS

Isolation and identification of bacteria. Altogether, 10 cultures were isolated, purified, and studied. These organisms were identified according to *Bergey's Manual*. The results of various tests performed are given in Table 1. Four strains were identified as *Aerobacter aerogenes*, two as *Klebsiella pneumoniae*, and one as *Serratia kiliensis*. All of these cultures were injected intraperitoneally into mice; 0.5 ml of a 50 Klett unit cell suspension was used per mouse. Only the *K. pneumoniae* killed mice in 3 days, and examination of the lungs revealed consolidation of lobes with friable and granular appearance. Colonial growth of this organism stringed out when touched with a needle. *Klebsiella* was also identified by positive Quellung reaction with the use of *Klebsiella* polyantiserum (Difco; used according to the method described in the "Difco Supplementary Literature,

TABLE 1. Cultural characteristics of the organisms isolated^a

Organism	Isolate no. and source	Gram stain	Motility	Gelatin hydrolysis	Glucose	Lactose	Sucrose	Mannitol	Glycerol	Litmus milk	Methyl red test	Voges-Proskauer test	Indole	Citrate utilization	H ₂ S production	Urea hydrolysis	Uric acid	Nitrate reduction	Pigment	Capsule
<i>Aerobacter aerogenes</i>	1, 2, 4, 6; chicken droppings	-	-	-	(A)	(A)	(A)	(A)	(A)	(A) R	-	+	-	+	+	+	+	+	-	+
<i>Klebsiella pneumoniae</i>	8, 10; sewage	-	-	-	(A)	(A)	(A)	A	(A)	A	-	+	-	+	-	+	+	+	-	+
<i>Serratia kiliensis</i>	20; sewage	-	+	+	(A)	(A)	(A)	(A)	(A)	A	+	-	-	+	+	+	+	+	+	-
<i>Pseudomonas fluorescens</i>	12; frog waste	-	+	+	A	-	A	A	-	a	-	-	-	+	-	+	+	+	+	-
<i>P. aeruginosa</i>	25; sewage	-	+	+	-	-	-	-	-	aPR	-	-	-	+	-	+	+	+	+	-
<i>Bacillus</i> sp.	13; contaminant	V	+	+	A	-	A	A	A	AP	+	-	-	-	-	+	+	-	-	+

^a Symbols: A = acid; (A) = acid and gas; a = alkaline; P = peptonization; R = reduction; V = variable; - = negative or no change; + = positive.

1962"). It also formed a much thicker capsule than *A. aerogenes*. *S. kiliensis* was easily identified by its orange-red pigment in agar medium, by the fact that it was readily soluble in ethyl alcohol, and by its gas production from various sugars, its Voges-Proskauer reaction, and its methyl red reaction. In Nutrient Agar, *P. aeruginosa* produced typical blue-green pigment soluble in chloroform. This organism was also able to grow at 42 C. The pigment of *P. fluorescens* was faintly yellow-green and insoluble in chloroform, and this organism did not grow at 42 C; best growth was obtained at 25 C. Also, it produced acid in glucose, sucrose, and mannitol fermentation tubes. The *Bacillus* species was identified by its ability to grow aerobically and to form spores. Further identification of this species has not been successful.

Growth studies. All of the isolated cultures grew well in the usual medium containing uric acid and glucose (Table 2). Colorimetric tests for the presence of residual glucose (7) at the end of the growth period were negative in all cases. When glucose was omitted from this medium, growth was poor for all organisms except *P. fluorescens*; there was no growth of *P. fluorescens* without glucose (Table 2). The inocula for the isolated organisms were obtained from cultures grown in the medium containing $(\text{NH}_4)_2\text{SO}_4$ and glucose, and inocula for laboratory cultures were taken from the respective growth media. In all cases, the inocula used for growth and adaptation studies were washed once in phosphate-buffered solution (pH 7.1; 0.05 M). A loopful of culture from a 70 to 100 Klett unit washed suspension was used as inoculum. Of the laboratory stock cultures, *A. aerogenes*, *B. subtilis*, *P. aeruginosa*, *P. fluorescens*, and *S. marcescens* showed good growth in uric acid medium with glucose; when glucose was omitted, they showed poor growth (Table 2). *Mycobacterium phlei* showed about the same amount of growth in medium containing uric acid, with or without glucose. Two other laboratory stock cultures, *Aeromonas hydrophila* and *Proteus mirabilis* grew poorly in medium containing both glucose and uric acid. Another 15 laboratory stock cultures, namely, *Acetobacter xylinum* (MC 1), *Alcaligenes faecalis* (MC 6), *Arthrobacter globiformis* (MC 8), *Azotobacter chroococcum* (ATCC 9043), *B. stearothermophilus* (ATCC 12980), *Chromobacterium violaceum* (MC 22), *Corynebacterium pseudodiphtheriticum* (MC 28), *Escherichia coli* (ATCC 11775), *Gaffkya species* (MC 39), *Micrococcus agilis* (MC 44b), *Photobacterium phosphoreum* (ATCC 11040), *Staphylococcus* species, *Streptococcus agalactiae* (ATCC 13813), *S. lactis* (MC 82), and a β -hemolytic *Streptococcus*, when inoculated into

TABLE 2. Utilization of uric acid as a nitrogen source for growth by various organisms^a

Organism	Growth (μg of dry wt/ml)	
	Uric acid and glucose	Uric acid only
<i>Aerobacter aerogenes</i> (MC 3).....	462 ^b	36
<i>Aeromonas hydrophila</i> (ATCC 9071).....	27	0
<i>Bacillus subtilis</i> (ATCC 6051).....	402	78
<i>Mycobacterium phlei</i> (MC 53).....	23	23
<i>Proteus mirabilis</i> (ATCC 9240)....	31	0
<i>Pseudomonas aeruginosa</i> (ATCC 10145).....	480	114
<i>P. fluorescens</i> (ATCC 11250).....	406	0
<i>Serratia marcescens</i> (MC 75).....	180	75
<i>A. aerogenes</i> (1, 2, 4, 6).....	398	70
<i>Klebsiella pneumoniae</i> (8, 10)....	457	84
<i>S. kiliensis</i> (20).....	429	93
<i>P. fluorescens</i> (12).....	420	0
<i>P. aeruginosa</i> (25).....	463	122
<i>Bacillus</i> species (13).....	412	92

^a The medium contained the usual minerals, 1% glucose (when added), and uric acid (1.5 mg/ml), and the organisms were grown up to 3 days. ATCC = American Type Culture Collection; MC = Midwest Culture Service.

^b These values were obtained from standard curves by converting the Klett turbidity units to dry weight.

the uric acid medium with or without glucose, did not grow.

Repeated transfer of all of the isolated cultures except *P. fluorescens*, and of the laboratory stock cultures of *A. aerogenes*, *P. aeruginosa*, *S. marcescens*, and *B. subtilis*, into the medium containing uric acid and no glucose, showed their ability to grow with uric acid as the only source of carbon, nitrogen, and energy (Table 3). In some cases, a slight and perhaps insignificant increase in growth of second transfer was observed. To rule out the occurrence of mutants, these cultures were recultivated in uric acid-free medium; subsequently, they required a typical time lag for adaptation for utilization of uric acid. In each transfer, the culture was allowed to incubate in a shaker at 30 C for 3 days, and then other flasks containing the same medium in the same volumes were inoculated with inocula of the same size. After the fifth transfer, the cultures were rechecked and were found to be pure.

The isolated organisms that were capable of utilizing uric acid were further tested for their ability to utilize adenine, guanine, hypoxanthine, xanthine, and allantoin. Each of these compounds

TABLE 3. Growth of cultures with uric acid as the only source of carbon, nitrogen, and energy^a

Organism	Transfer no.				
	1	2	3	4	5
<i>Aerobacter aerogenes</i> ^b (1, 2, 4, 6).....	99 ^c	116	112	102	100
<i>Klebsiella pneumoniae</i> (8, 10).....	110	120	118	126	107
<i>Serratia kiliensis</i> (20).....	68	91	86	89	85
<i>S. marcescens</i> (MC 75).....	78	71	64	66	66
<i>Pseudomonas aerugi- nosa</i> ^b (25).....	91	120	120	116	127
<i>Bacillus</i> sp. (13).....	80	111	100	122	115
<i>B. subtilis</i> (ATCC 6051).....	82	95	102	98	92
<i>Mycobacterium phlei</i> (MC 53).....	23	30	26	20	24

^a The medium contained the usual minerals and uric acid (1.5 mg/ml); the organisms were grown up to 3 days.

^b ATCC cultures of the same name gave almost identical results.

^c The amount of growth in micrograms of dry weight per milliliter.

supported good growth in the medium containing glucose. When glucose was omitted, growth was slight to poor. A comparison of growth on these compounds suggested that xanthine supports the most growth and allantoin the least.

Induction of adaptive enzymes. The lag in the disappearance of the uric acid with washed cells, grown in $(\text{NH}_4)_2\text{SO}_4$ medium, of *A. aerogenes*, *K. pneumoniae*, *S. kiliensis*, *P. fluorescens*, *P. aeruginosa*, the *Bacillus* species, and the laboratory stock cultures of *B. subtilis* (ATCC 6051) and *S. marcescens* (MC 75) showed that the ability of these organisms to decompose uric acid was not a constitutive property, but an adaptive one. Results of typical experiments are illustrated in Fig. 1 and 2. There was a time lag of 1 to 6 hr before the uric acid started to disappear from the medium at an appreciable rate, and *P. aeruginosa* required overnight (18 hr) to deplete the entire amount of uric acid. *Bacillus* species showed the shortest lag period (1 hr), and this organism also degraded the entire amount of uric acid in 4 hr. The lag period disappeared when the organisms were grown in uric acid instead of $(\text{NH}_4)_2\text{SO}_4$. An exception to this finding was noted in *P. aeruginosa*. This organism showed a time lag of 2 hr even when it was grown in uric acid. Both *S. kiliensis* and *S. marcescens* gave results almost identical to those with *P. fluorescens*.

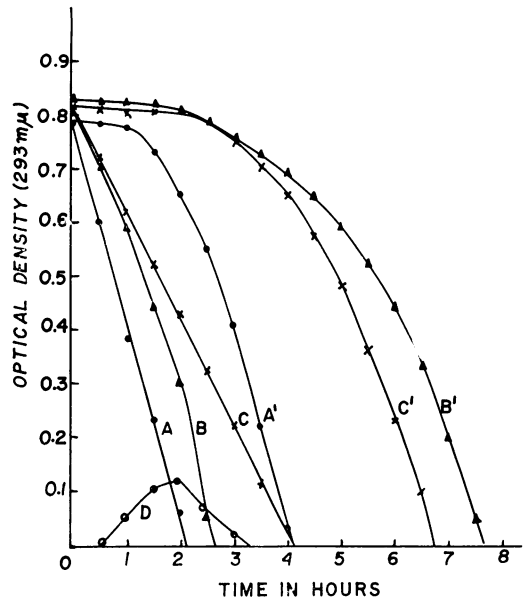


FIG. 1. Optical density of the uric acid medium. Curves A, B, and C: organisms grown in medium containing uric acid (preinduced), and curves A', B', and C': organisms grown in $(\text{NH}_4)_2\text{SO}_4$ (uninduced). Abscissa: time after adding inducer (uric acid). A and A', *Bacillus* species; B and B', *B. subtilis*; C and C', *Klebsiella pneumoniae*. Curve D represents the intracellular accumulation of uric acid by *Bacillus* sp., when grown in uric acid medium.

With the exception of the *Bacillus* species, none of the bacteria studied showed any intracellular accumulation of uric acid from the medium. Intracellular accumulation of uric acid in the *Bacillus* species reached a maximum at about 2 hr, following induction in the uric acid medium (Fig. 1, curve D).

Experiments identical to those used for induction of adaptive enzymes, but with no uric acid present, were done. In all of these cases, the supernatant fluid showed no absorption at any time (up to 8 hr) at 293 nm. This was considered to be evidence that no other material absorbing at this wavelength was present.

DISCUSSION

In this investigation, the various species isolated and identified as *A. aerogenes*, *K. pneumoniae*, *S. kiliensis*, *P. aeruginosa*, and *Bacillus* species had the ability to grow with uric acid as the only source of carbon, nitrogen, and energy. Successive transfer of the cultures to the uric acid medium always yielded consistent growth comparable to the first transfer. This could be taken as evi-

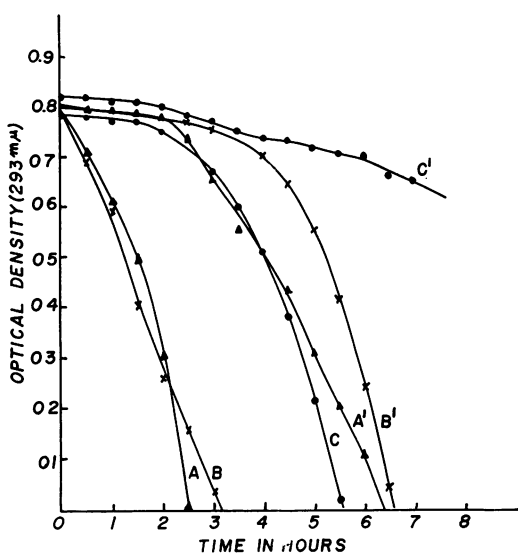


FIG. 2. Optical density of the uric acid medium. Curves A, B, and C: organisms grown in medium containing uric acid (preinduced), and curves A', B', and C': organisms grown in $(NH_4)_2SO_4$ (uninduced). Abscissa: time after adding inducer (uric acid). A and A', *Aerobacter aerogenes*; B and B', *Pseudomonas fluorescens*; C and C', *P. aeruginosa*.

dence that the uric acid is undoubtedly utilized by these organisms as a source of carbon, nitrogen, and energy. The culture identified as *P. fluorescens*, however, did not grow with uric acid as sole source of carbon, nitrogen, and energy. It could utilize uric acid as a nitrogen source when another carbon source, such as glucose, was present.

Of the laboratory cultures, *A. aerogenes*, *B. subtilis*, *P. aeruginosa*, and *S. marcescens* were also found to be capable of growing with uric acid as the only source of carbon, nitrogen, and energy. Cultures of *M. phlei* yielded very poor growth.

When glucose was added to uric acid medium, glucose was depleted by all of the organisms studied. Thus, these organisms differ from *C. acid-iurici* and *C. cylindrosporium* (2), which grew with uric acid as the source of carbon and nitrogen; glucose, when added, was not attacked by these two bacteria even after 5 days of incubation. Adenine, guanine, hypoxanthine, xanthine, and allantoin each supported poor growth of the organisms studied. Addition of glucose to the medium with the above substrates yielded good growth in every case. Thus, it seems that all of the purines studied support growth of the organisms in a similar fashion. This study confirms the

finding (1) that certain *Pseudomonas* species can use uric acid as the sole source of carbon, nitrogen, and energy. In addition, for the first time *A. aerogenes*, *K. pneumoniae*, *S. marcescens*, *S. kiliensis*, *Bacillus* species, and *B. subtilis* are reported to be capable of growth with uric acid as the sole source of carbon, nitrogen, and energy. *M. phlei* is capable of only limited growth with uric acid. The normal intestinal flora include *A. aerogenes* and *Bacillus* species. Hence, these results, along with those of other studies with *Clostridium* (2, 8) and yeast (9, 10), support the hypothesis that uric acid, if administered orally to humans, is decomposed by intestinal bacteria (6, 11). However, the importance of aerobic decomposition of uric acid in the intestinal tract by these organisms has not yet been ascertained.

This investigation also demonstrated that the ability of *A. aerogenes*, *K. pneumoniae*, *P. aeruginosa*, *P. fluorescens*, *S. marcescens*, *S. kiliensis*, *B. subtilis*, and *Bacillus* species to degrade uric acid is an induced property. Lack of intracellular accumulation of the uric acid in all but *Bacillus* species is indicative of their ability to degrade uric acid as soon as it is transported into the cell. *Bacillus* species accumulated a small amount of intracellular uric acid and is somewhat comparable to *Torulopsis utilis* (9), which shows a large intracellular accumulation of uric acid.

Preparation of cellular extracts active in the degradation of uric acid and isolation of the enzymes and the intermediates involved in the degradation of uric acid are presently being investigated in this laboratory.

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