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Uric Acid Degradation by *Bacillus fastidiosus* Strains

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Seven *Bacillus* strains including one of the original *Bacillus fastidiosus* strains of Den Dooren de Jong could grow on urate, allantoin, and, except one, on allantoate. No growth could be detected on adenine, guanine, hypoxanthine, xanthine, and on degradation products of allantoate. Some strains grew very slowly in complex media. The metabolic pathway from urate to glyoxylate involved uricase, S(+)-allantoinase, allantoate amidohydrolase, S(−)-ureidoglycolase, and, in some strains, urease.

In 1929 Den Dooren de Jong (4) described a fastidious *Bacillus* species that was able to grow only on urate and allantoin. The bacterium was called *Bacillus fastidiosus*. Similar bacteria were recently isolated by Leadbetter and Holt (9, 12), Claus (according to Kaltwasser [10]), and Mahler (13). The characteristics of some of these strains and four new isolates are compared in this study.

Various routes of uric acid degradation by bacteria are known. Except for *Veillonella alcalescens* (30), *Clostridium cylindrosporum*, and *Clostridium acidiurici* (1), all pathways involve allantoin, allantoate, ureidoglycolate, glyoxylate, and urea as intermediates. The enzymes involved and the optical isomers of the various intermediates are different in the various bacteria studied (24). The present paper deals with the uric acid degradation by seven *B. fastidiosus* strains.

MATERIALS AND METHODS

Bacterial strains. *B. fastidiosus* SMG 83 was obtained from H. Kaltwasser (10), Department of Microbiology, University of Saarland, Saarbrücken, Germany. *B. fastidiosus* strain 1051, an original isolate of Den Dooren de Jong (4), was supplied by T. O. Wikén, Department of Microbiology, Technological University, Delft. *B. fastidiosus* strain NCIB 10372, an isolate of J. L. Mahler (13), was obtained from NCIB. Four other strains were isolated from soil by incubation of different soil samples (0.5 g) in 100 ml of medium containing 1 g of urate and 0.1 g of K_2HPO_4 in tap water (pH 6.7). Incubation was at 37 C for 3 to 4 days with rotatory shaking in a New Brunswick model G25 environmental incubator (200 rpm). Culture samples were heated for 10 min at 80 C and plated on the same medium containing 1.2% agar. The strains were isolated by four transfers on plates containing this medium. Strains growing well on 3% Trypticase soy broth (TSB, BBL) within 4 days were discarded. The resulting strains were divided into four groups on the basis of their sensitivity to various antibiotics, morphological appearance, colony form, and color. Of each group one strain, coded A.2, C.4, C.6.A, or E.1, was used in this study. The strains were preserved at 4 C after growth for 2 days at 37 C on plates or slants containing 0.4% uric acid or 0.6% allantoin, 0.3% TSB, 1.2% agar, and the trace element buffer according to Kaltwasser (10). Transfers were made every 3 to 4 weeks.

Media used in growth tests. Growth experiments revealed that the medium used during the isolation was suboptimal. Trace elements and in some cases small amounts (0.3 to 0.4%) of TSB or brain heart infusion (BHI, Oxoid) improved growth.

Growth tests were performed in standard media containing one of the following compounds in trace element phosphate buffer (pH 7.2) (10): 0.3% purine (adenine, guanine, hypoxanthine, or xanthine), 0.4% uric acid, 0.6% allantoin, 0.6% sodium allantoate, or 0.5% sodium ureidoglycolate. The media were sterilized at 121 C except for allantoate and ureidoglycolate, which were sterilized by Seitz filtration. Allantoate is unstable at high temperatures (27), whereas ureidoglycolate is hydrolyzed slowly also at room temperature (20). Media containing the latter compound were used immediately after preparation.

To determine the generation time on urate, a clear medium was used consisting of 0.3 g of uric acid dissolved in 90 ml of 0.1 N NaOH and then neutralized to pH 7.5 to 7.6 with 10% KH_2PO_4 solution while the temperature was kept above 30 C. Trace elements were added to the same concentrations as used in the standard media. Water was added to obtain a volume of 110 ml. This medium was sterilized by Seitz filtration.

The presence of flagella was studied by electron microscopy after negative straining with uranyl acetate of washed preparations of cells taken from the mid-exponential phase of an allantoin-TSB culture.

Photographs of cells were made according to the interference contrast method of Nomarski (14) with a Leitz microscope.

Preparation of cell suspensions. Cells were grown in standard media (50 ml) containing urate, allantoin, or allantoate. The media were heavily inoculated and

incubated overnight at 37 C with rotatory shaking (200 rpm). The cultures obtained were used to inoculate 1.5 liters of media, which were incubated under similar conditions. Cells were harvested by centrifugation, washed twice with the buffered trace element solution, and suspended in 75 ml of this solution. The suspensions were stored at −20 C.

Cell-free extracts. Cells were disrupted, while chilled at 0 C, in a 100-W MSE ultrasonic disintegrator for 75 s with intermittent cooling. The sonicated suspension was centrifuged at 4 C for 20 min at 100,000 × *g*. The supernatant was stored at −20 C. Extracts used for allantoinase (allantoin amidohydrolase: EC 3.5.2.5) assays were stored at 4 C, since the enzyme appeared to be more stable at this temperature.

Enzymatic procedures. Guanine deaminase (guanine aminohydrolase; EC 3.5.4.3) and adenine deaminase (adenine aminohydrolase; EC 3.5.4.2) were assayed by measurement of the ammonia produced by extracts incubated with guanine present at the saturated concentration or adenine present at a concentration of 3.0 mM, respectively, in 40 mM phosphate buffer (pH 7.2) at 30 C. Ammonia was determined by the phenol-hypochlorite method (15).

Xanthine dehydrogenase (xanthine:oxidized nicotinamide adenine dinucleotide oxidoreductase; EC 1.2.1.37) was assayed as described by Watt (29), but the incubation volume was 2 ml and the oxidized nicotinamide adenine dinucleotide concentration was 0.6 mM.

Uricase (urate:oxygen oxidoreductase; EC 1.7.3.3) activity was measured according to Mahler (13); 0.2-ml samples of the reaction mixture were diluted in 3.8 ml of 0.1 N HCl. Allantoinase, allantoate amidohydrolase (allantoate amidinohydrolase [decarboxylating]; EC 3.5.3.9), and ureidoglycolase (ureidoglycolate urea-lyase; EC 4.3.2.3) were determined by measurement of the amounts of allantoate, ureidoglycolate, and glyoxylate, respectively, according to the differential glyoxylate analysis (28); urease (urea amidohydrolase; EC 3.5.1.5) was measured according to either the phenol-hypochlorite method (15) or the glutamate dehydrogenase assay, according to Vogels et al. (26), but 0.05 ml of glutamate dehydrogenase in glycerol was added instead of 0.1 ml.

Polarimetric measurements. Polarimetric measurements were performed at 30 C in a Perkin-Elmer model 141 polarimeter at 365 nm in a 10-cm quartz cuvette. Optical rotations were followed as a function of time for allantoinase, allantoate amidohydrolase, and ureidoglycolase. Uricase assays were performed with clear solutions containing 18 μmol of urate per ml of phosphate buffer, which were prepared as described before, but no trace elements were added. Allantoinase was tested in an incubation mixture containing, per milliliter, 50 μmol of RS-allantoin and 270 μmol of triethanolamine-hydrochloride (pH 7.8). Allantoate amidohydrolase was assayed in mixtures containing, per milliliter, 100 μmol of sodium allantoate, 270 μmol of diethanolamine-hydrochloride (pH 8.8), 0.25 μmol of $MnSO_4$, and 6.8 μmol of reduced glutathione. The enzyme was activated by acid pretreatment (23) at pH 2.0 for 20 s at 0 C. Ureidoglycolase was measured in mixtures containing, per milliliter, 75 μmol of sodium RS-ureidoglycolate, 0.75 μmol of $MnSO_4$, and 200 μmol of triethanolamine-hydrochloride (pH 7.5).

R- and S-allantoin and R- and S-ureidoglycolate are defined according to the nomenclature of Cahn et al. (2, 3). R-allantoin is equivalent to (−)-allantoin (18), and R-ureidoglycolate is equivalent to (+)-ureidoglycolate (17). The enzymatic activity was calculated from the polarimetric data by use of the values of the molar rotations, which are −508° for R-allantoin (18) and +35° for R-ureidoglycolate (17) under the conditions used.

The specific activities of all enzymes are given as micromoles of substrate converted per minute per milligram of protein.

Chromatography. Urate and its degradation product allantoin were separated by thin-layer chromatography on polyethyleneimine-cellulose F (Merck). The solvent system consisted of 4 parts 0.15 M NaCl and 1 part 95% ethanol (8).

RESULTS

Description of the strains. The cells of the seven strains of *B. fastidiosus* were rods with a size of 2.0 to 6.6 by 1.1 to 1.7 μm (Table 1), had round ends, and were motile (except strain 1051) by means of peritrichous flagella and gram positive when tested with cells taken from a young

TABLE 1. *Properties of Bacillus fastidiosus strains*

Strain	Cells		Mean length of spores (μm)	Colony form[b]	Origin
	Mean length (μm)[a]	Mean width (μm)[a]			
A.2	4.3	1.55	2.0	I	
C.4	2.7	1.15	1.3	Pu	
C.6.A	2.7	1.15	1.3	Ro-Pu	
E.1	2.7	1.55	1.3	Pu	
SMG 83	4.7	1.55	1.8	Rh-Ro	D. Claus
1051	6.6	1.70	2.0	Rh	L. E. Den Dooren de Jong
NCIB 10372	2.0	1.15	1.0	Pu	J. L. Mahler

[a] As measured during exponential growth.
[b] Pu, Punctiform; Ro, round; I, irregular; Rh, rhizoid.

culture. Oval spores were formed with a size of 1.0 to 2.0 by 0.8 to 1.0 μm.

Sporangia were not appreciably swollen. Five strains formed central spores, whereas two strains (SMG 83 and 1051) formed terminal spores. The bacteria were aerobic and did not grow under anaerobic conditions on urate or allantoin medium at 37 C. The form of the white to pale-yellow colonies is given in Table 1. Figure 1 shows photographs of cells of one of the original strains of Den Dooren de Jong, strain 1051, and of strain C.4 taken from the exponential phase. Strain 1051 exhibited a strong tendency toward autolysis.

Growth in various media. Media found to support growth are given in Table 2. Urate and its degradation products, allantoin and allantoate, yielded abundant growth except for strain C.4, which was not able to grow on allantoate. Rich media such as TSB, BHI, and casein hydrolysate (enzymatic) (all 3%) allowed only very limited or no growth. The limited growth was not due to the presence of urate, allantoin, or allantoate since the amounts of these components were less than 0.03% as determined on a dry-weight basis. Strain NCIB 10372 needed some components from complex media for growth on urate, allantoin, and allantoate. Adenine, guanine, hypoxanthine, and xanthine could not be used for growth by any of the strains under conditions similar to those used in the tests with urate and its degradation products. No growth was observed in Seitz-filtered media containing 0.3% NH_4Cl and 0.2% sodium glyoxylate, sodium glycolate, sodium fumarate, or sodium potassium tartrate in trace element phosphate buffer (pH 7.2). Addition of 0.3% TSB did not improve the growth.

Generation time in various media. The generation time as judged by the increase of optical density at 600 nm is given in Table 3. The growth rates in minimal media containing urate or allantoin were higher than in media containing allantoate except for strain 1051. TSB (0.3%) supported growth only very weakly but had a stimulating effect on growth, especially for strain NCIB 10372. Some strains grew to some extent on 3% TSB. Similar results were obtained when TSB was replaced by BHI.

Degradation of urate. The conversion of urate into allantoin by crude extracts of *B. fastidiosus* cells resulted in a positive optical rotation of the incubation mixture when assayed at or below pH 7.6. These results indicate that S(+)-allantoin is the product of urate degradation, but no quantitative correlation could be established between the disappearance of urate and the observed optical rotation, since allantoin was subject to a rapid racemization (25) even at these pH values, which are suboptimal for uricase action. The presence of allantoin in the reaction mixtures was demonstrated by chromatographic means. Per mol of urate, 0.45 mol of oxygen was consumed (Fig. 2). A similar result was obtained by Kaltwasser (10).

Degradation of allantoin. On incubation of crude extracts with RS-allantoin, allantoate was formed and simultaneously a negative optical rotation was observed in the incubation mixture (Fig. 3). The results indicate that allantoinase reacts specifically with S(+)-allantoin.

Degradation of allantoate. Two enzymes are known to be involved in the degradation of allantoate by bacteria. Allantoicase (allantoate amidinohydrolase; EC 3.5.3.4) is present in a number of *Pseudomonas* species, whereas various other bacteria contain allantoate amidohydrolase (24). Both enzymes produce ureidoglycolate. However, allantoicase forms urea whereas the allantoate amidohydrolase reaction results in the formation of NH_3 and CO_2 from allantoate.

In all instances tested so far (22, 23), allantoate amidohydrolase was present in a rather inactive form in crude bacterial extracts. The activity could be enhanced considerably by pretreatment at low pH values or at pH about 6 in the presence of suitable chelating substances (22). The enzyme of *B. fastidiosus* behaved this way and converted allantoate to ammonia and ureidoglycolate in a ratio of approximately 2:1 (Fig. 4). It is obvious that S(−)-ureidoglycolate was produced. The production of ammonia was not due to a combined action of allantoicase and urease since ammonia was also formed by the extracts of the four strains of *B. fastidiosus* that do not contain urease. The results obtained with the crude extract of the urease-negative strain E.1 are depicted in Fig. 4.

Degradation of ureidoglycolate. Ureidoglycolate is converted into glyoxylate and urea by ureidoglycolase (Fig. 5). The formation of urea could be established by performing the reaction with a crude extract of a urease-negative *B. fastidiosus* strain, followed by measurement of ammonia with glutamate dehydrogenase in the presence or absence of jack bean urease. The ammonia-glyoxylate and urea-glyoxylate ratios were 2 and 1, respectively. Polarimetric measurements performed with RS-ureidoglycolate demonstrated that S(−)-ureidoglycolate is the substrate of ureidoglycolase. Under the conditions applied, R(+)-ureidoglycolate did not accumulate in stoichiometrical amounts due to racemization (17).

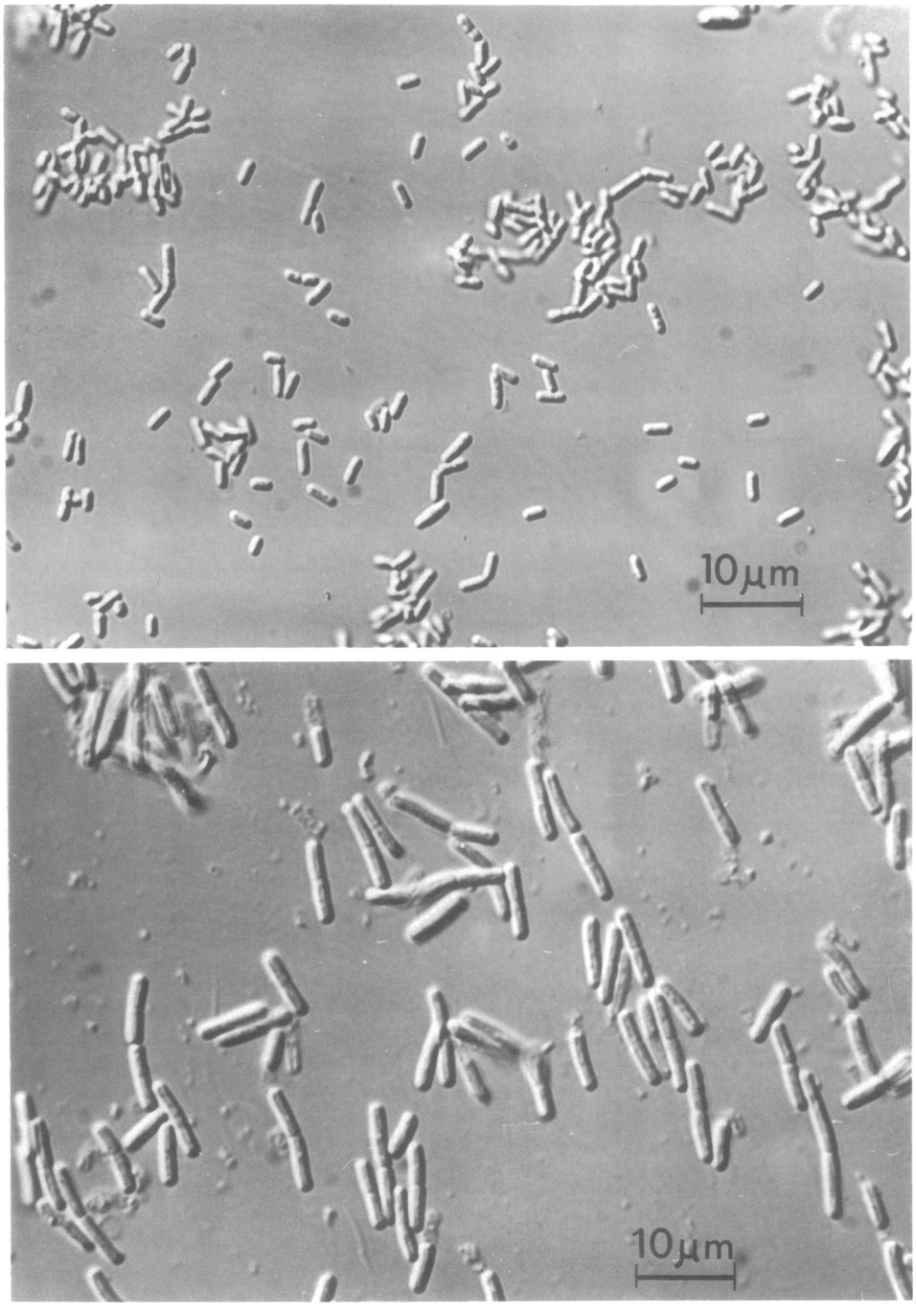

FIG. 1. *Allantoin-TSB-grown cells of two B. fastidiosus strains. (A) B. fastidiosus C.4; (B) B. fastidiosus 1051.*

Degradation of urea. Urease was present only in *B. fastidiosus* strains A.2, SMG 83, and 1051 grown on urate, allantoin, or allantoate. The presence of urease in strain SMG 83 was previously reported by Kaltwasser (10).

Induction of enzymes. Uricase was induced by urate in all strains (Table 4). This result agrees with those reported previously by Kaltwasser (10) for *B. fastidiosus* SMG 83. Small amounts of the enzyme were present in *B.*

TABLE 2. *Growth capabilities of Bacillus fastidiosus strains on various media*

Medium[a]	Strain[b]						
	A.2	C.4	C.6.A	E.1	SMG 83	1051	NCIB 10372
0.3% Urate	++	+	++	++	++	++	−
0.3% Urate + 0.3% TSB	++	++	++	++	++	++	++
0.6% Allantoin	++	+	++	++	++	++	−
0.6% Allantoin + 0.3% TSB	++	++	++	++	++	++	++
0.6% Allantoate	++	−	++	++	++	++	−
0.6% Allantoate + 0.3% TSB	++	−	++	++	++	++	++
3% TSB	±	−	±	±	±	+	+
3% BHI	±	−	±	+	±	+	+
3% Casein hydrolysate	−	−	−	±	±	±	±

[a] All media are described in the text. Incubation was at 37 C. Liquid media were shaken in a rotatory shaker (200 rpm), and growth was followed by measurement of the absorbancy at 600 nm.

[b] −, No growth; ±, scarce growth; +, reasonable growth; ++, abundant growth.

TABLE 3. *Generation time during exponential growth of Bacillus fastidiosus strains on various media*[a]

Strain	Generation time (min)					
	Urate + 0.3% TSB	Urate	Allantoin + 0.3% TSB	Allantoin	Allantoate	0.3% TSB
A.2	40-45	60-70	40-45	45-50	90	—[b]
C.4	65-70	65-70	70-75	130-150	—	—
C.6.A	45-55	65-70	45-50	65-70	100	>300
E.1	50-60	60-65	55-60	50-60	150	—
SMG 83	45-55	50-60	40-45	45-55	75	—
1051	60-65	65-70	35-45	55-60	45-50	>300
NCIB 10372	75-85	—	90-95	—	90-95[c]	>300

[a] Standard media as described in the text were inoculated with cultures in the late exponential phase pregrown on allantoin-TSB medium. Incubations were at 37 C in a rotatory shaker (200 rpm) in a 100-ml Erlenmyer flask.

[b] —, No growth.

[c] 0.3% TSB was added.

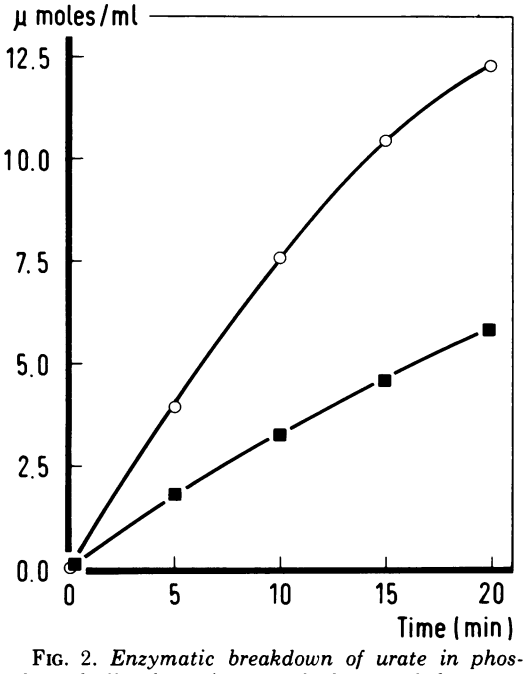

FIG. 2. *Enzymatic breakdown of urate in phosphate buffer by uricase and the coupled oxygen consumption. The reaction mixture contained, per milliliter, 15 µmol of urate, 80 µmol of K_2HPO_4-NaH_2PO_4 buffer (pH 7.5), and an amount of crude extract of Bacillus fastidiosus SMG 83 equivalent to 0.15 mg of protein. Disappearance of urate was measured spectrophotometrically at 283 nm after dilution in 0.1 N HCl. Oxygen consumption was measured manometrically in a Gilson differential respirometer. Symbols: O, Amount of urate disappeared; ■, amount of oxygen consumed.*

fastidiosus strain C.4 and NCIB 10372 after growth in allantoin or, in case of the latter strain, in allantoate media. Growth of these strains for 30 to 35 generations in allantoin medium did not reduce the specific activities of uricase below the indicated values. Allantoinase, allantoate amidohydrolase, and ureidoglycolase were present in cells growing in urate, allantoin, or allantoate medium.

Although none of the strains could grow on the purines adenine, guanine, hypoxanthine, and xanthine under the conditions applied for growth on urate and its degradation products, cells grown on urate or on allantoin were tested for the presence of the enzymes adenine deaminase, guanine deaminase, and xanthine dehydrogenase. None of these enzymatic activities could be detected in the crude extracts of the seven strains.

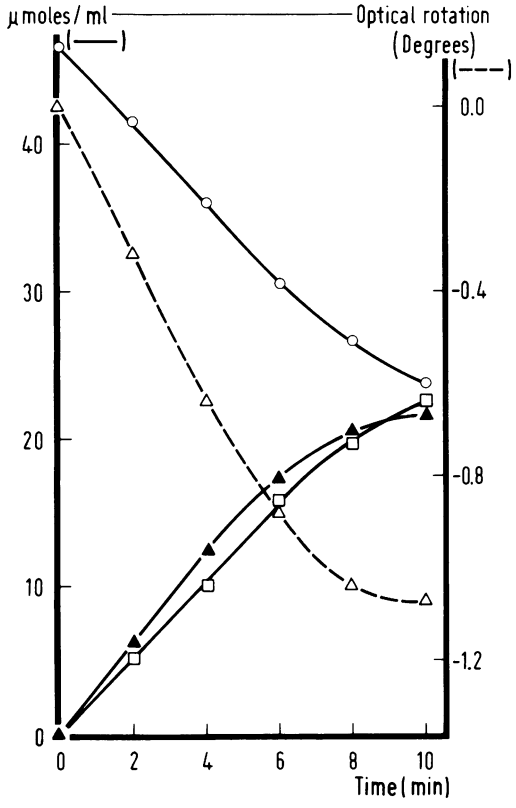

FIG. 3. *Enzymatic conversion of allantoin by allantoinase. The reaction mixture contained, per milliliter, 46.5 µmol of allantoin, an amount of crude extract of B. fastidiosus SMG 83 equivalent to 0.35 mg of protein, and 200 µmol of triethanolamine-hydrochloride (pH 7.5). Symbols: ○, Allantoin present; □, allantoate formed; ▲, R-allantoin accumulated during the reaction; △, optical rotation.*

DISCUSSION

The strains of *B. fastidiosus* studied in this report have a number of properties in common, but striking differences must be noted that allow the distinction of the strains. According to the description of Den Dooren de Jong, *B. fastidiosus* forms rhizoidal colonies (4). This appears to be true only for his isolate. In contrast to the observation of Den Dooren de Jong, all strains are gram positive; Leadbetter and Holt (12) already pointed out that Den Dooren de Jong may have used a preparation of old cells. We report a strong tendency to autolyze for one of his strains that can also account for the negative result of Den Dooren de Jong.

The most striking common property of the strains is their ability to grow well only on urate, allantoin, and (all but one strain) allantoate. However, this point deserves two comments: (i) the requirement for these substrates appears not to be absolute for all strains, since some of the strains can grow slightly on complex media; and (ii) the property to degrade urate, allantoin, or allantoate appears not to be confined to *B. fastidiosus* among the *Bacillus* species. *B. megaterium* (19), *B. guano* (19), *B. hollandicus* (19) (later identified as *B. brevis*), *B. polymyxa* (L. E. Den Dooren de Jong, dissertation, Technological University, Delft, 1926), and *B. subtilis* (6, 21; Den Dooren de Jong, dissertation) can use urate as a nitrogen source but do not grow in a medium containing this substance as the sole substrate. Rouf and Lomprey (16) reported growth

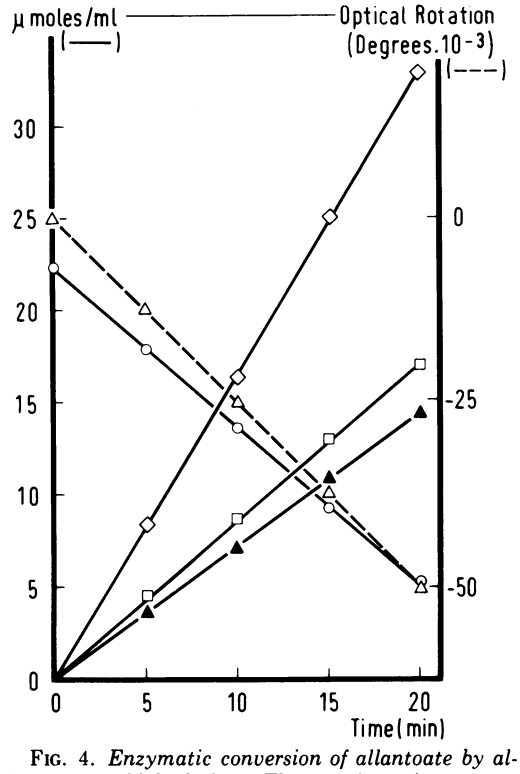

FIG. 4. *Enzymatic conversion of allantoate by allantoate amidohydrolase. The reaction mixture contained, per milliliter, 22 µmol of sodium allantoate, an amount of crude extract of B. fastidiosus E.1 equivalent to 0.37 mg of protein, 4.25 µmol of reduced glutathione, 0.085 µmol of $MnSO_4$, and 170 µmol of diethanolamine-hydrochloride (pH 8.8). The enzyme was pretreated at pH 1.8 in 0.05 M phosphoric acid for 20 s (0 C) and added to the reaction mixture at the start of the experiment. Assays with crude extracts not pretreated in this way yielded ureidoglycolate and ammonia at velocities equivalent to 1.5% of those with pretreated ones. Symbols: ○, Allantoate present; □, ureidoglycolate formed; ◇, ammonia formed; ▲, S-ureidoglycolate accumulated during the reaction; △, optical rotation.*

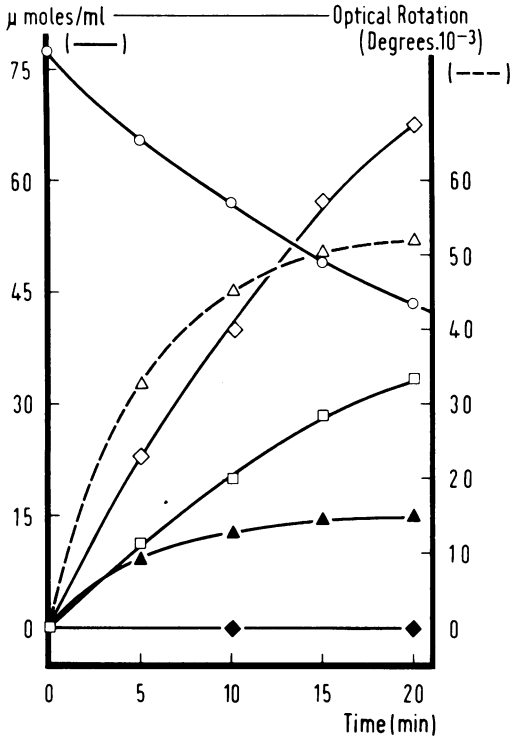

FIG. 5. *Enzymatic conversion of ureidoglycolate by ureidoglycolase. The reaction mixture contained, per milliliter, 77.5 μmol of sodium RS-ureidoglycolate, 0.38 μmol of $MnSO_4$, 100 μmol of triethanolamine-hydrochloride (pH 7.8), and an amount of crude extract of B. fastidiosus E.1 equivalent to 2.0 mg of protein. Ammonia was determined according to a glutamate dehydrogenase assay, both in the presence of jack bean urease (Serva; 0.13 mg per ml of assay mixture) and in its absence. Symbols: O, Ureidoglycolate present; □, glyoxylate formed; ◇, ammonia formed in the presence of urease; ◆, ammonia formed in the absence of urease; ▲, R-ureidoglycolate accumulated during the reaction; △, optical rotation.*

of *B. subtilis* and an unidentified *Bacillus* species on urate and allantoin, but these strains also use adenine, guanine, hypoxanthine, and xanthine for growth. Growth on urate appeared to be an adaptive characteristic, because the enzymes involved were induced after inoculation from a complex medium into an urate medium.

All strains of *B. fastidiosus* mentioned above decompose urate along the same catabolic pathway depicted in Fig. 6. In this figure the absolute configurations of the intermediates are given. Adenine, guanine, hypoxanthine, and xanthine are not broken down.

In 1965 Franke et al. reported first the formation of (+)-allantoin in the urate degradation by animal and plant uricases (7). The same compound appears to be formed by the uricase of *B. fastidiosus*. Allantoate is converted into ureidoglycolate, ammonia, and carbon dioxide by allantoate amidohydrolase. This enzyme is also found in *Escherichia coli*, *Proteus rettgeri*, *Pseudomonas acidovorans*, and *Streptococcus allantoicus* (23). The enzyme of *B. fastidiosus* produces S(−)-ureidoglycolate. The same reaction is catalyzed by the enzyme of *S. allantoicus*, whereas the enzyme of *P. acidovorans* forms R(+)-ureidoglycolate. All allantoate amidohydrolases have one striking property in common, since they are present in an inactive form in the bacterial extract and can be activated at low pH values or in the presence of chelating agents at about pH 6. The mechanism of this

TABLE 4. *Specific activities of the uricolytic enzymes in Bacillus fastidiosus strains*

Enzyme	Growth medium	Strain of *B. fastidiosus*						
		A.2	C.4	C.6.A	E.1	SMG83	1051	NCIB 10372
Uricase	Uric acid	8.1	9.0	3.9	4.1	10.3	10.8	10.2
	Allantoin	ND[a]	0.8	ND	ND	ND	ND	0.9
	Allantoate	ND	—[b]	ND	ND	ND	ND	1.0
Allantoinase	Uric acid	0.2	0.4	0.3	0.3	0.6	0.5	0.4
	Allantoin	0.5	0.7	0.3	0.8	1.3	0.3	1.5
	Allantoate	0.7	—[b]	0.7	0.3	0.7	0.9	0.6
Allantoate amidohydrolase	Allantoin	1.6	6.4	5.3	4.8	22.7	1.9	7.7
Ureidoglycolase	Allantoin	6.7	1.8	8.3	5.1	3.5	4.8	6.3
Urease	Uric acid	1.6	ND	ND	ND	3.3	4.5	ND
	Allantoin	3.9	ND	ND	ND	4.5	2.6	ND

[a] ND, Not detectable. The limits of detection in the enzyme assays were 0.05 for uricase and urease and 0.005 for allantoinase, allantoate amidohydrolase, and ureidoglycolase.

[b] Strain C.4 is unable to grow on media containing allantoate as the sole source of carbon, nitrogen, and energy even when supplemented with 0.3% TSB.

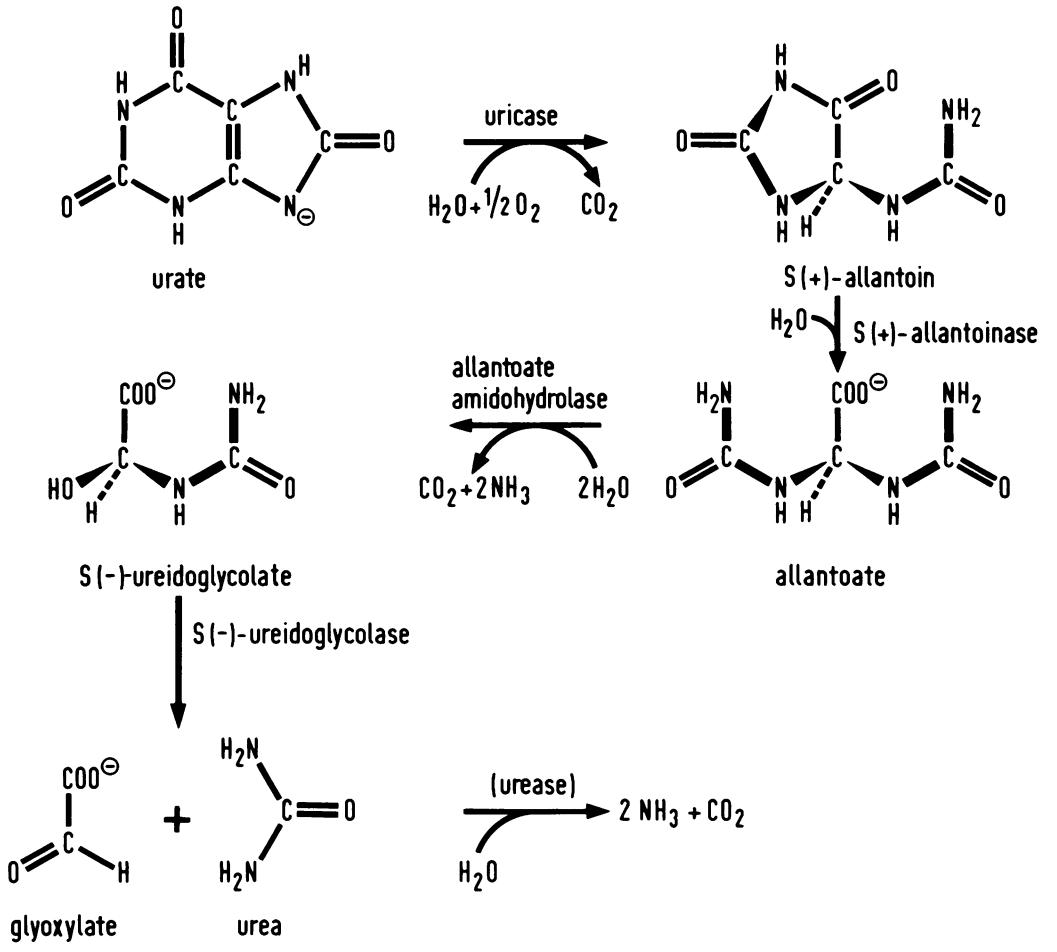

FIG. 6. *Catabolic pathway of uric acid to glyoxylate and urea in B. fastidiosus.*

activation was subject to an extensive study by Van der Drift and Vogels (22), but the physiological role of this property is still unknown.

B. fastidiosus C.4 does not grow in an allantoate medium but contains a normal set of enzymes and grows well on allantoin and urate. The results may reflect the presence of a transport barrier for allantoate in this strain.

The pathway of urate degradation found in *B. fastidiosus* may be due to the absence of the genetic information to produce this enzyme or to a strong repression of the enzyme production by ammonia as found in *P. aeruginosa* (5, 11).

The pathway of urate degradation found in *B. fastidiosus* differs only in some minor aspects from that found among other bacteria, and the results presented do not yield an explanation for the fastidious character of this bacterium.

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