Role of uricase in the triggering of germination of *Bacillus fastidiosus* spores

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The likelihood that uric acid was the only compound capable of triggering germination of *Bacillus fastidiosus* spores was reinforced by the finding that ureidoglycollic acid, urea, NH_4Cl , 2,8-dihydroxypurine and a combination of L-alanine and O-carbamoyl-D-serine were ineffective as germinants. Uric acid-triggered germination of *B. fastidiosus* was prevented by a range of inhibitors that also inhibited uricase activity in dormant spore extracts. O_2 uptake during germination started immediately after addition of uric acid, possibly as a consequence of the oxidation of uric acid by the enzyme uricase. Germination showed a dependence on uric acid concentration, with a relatively high K_m (4–5 mM). During the first 10 min of germination of heat-activated spores there was no detectable change in the number of spore-cortex reducing groups, indicating that selective cortex hydrolysis is not involved in the trigger mechanism of germination of *B. fastidiosus*. On the basis of the results, a model is proposed in which re-initiation of uricase activity is the mechanism by which *B. fastidiosus* spores are triggered to emerge from the dormant state.

Although a great variety of chemical and physical agents have been reported to trigger the germination of bacterial spores (Foerster & Foster, 1966; Gould, 1969), the molecular basis of the trigger mechanism has not been discovered. Studies on the effect of inhibitors of electron transport (Dring & Gould, 1975) and of pyruvate metabolism (Prasad, 1974), and analysis of mutants blocked in certain metabolic pathways, which have lost the ability to germinate (Wax *et al.*, 1967; Wax & Freese, 1968; Prasad *et al.*, 1972), led to the suggestion that triggering involved a metabolic energy-producing reaction. This hypothesis is now in doubt, however, after a series of experiments failed to detect any metabolism during the crucial triggering period, and demonstrated moreover that germination could be triggered by non-metabolizable analogues of the germinant (Wax & Freese, 1968; Rossignol & Vary, 1978; Scott & Ellar, 1978a). More recently a second hypothesis has been advanced, which proposes that the trigger event is a biophysical or purely hydrolytic reaction that must occur before energy-generating metabolism can commence

(Ellar, 1978a,b; Scott & Ellar, 1978b; Scott *et al.*, 1978; Rossignol & Vary, 1978; Vary, 1978). Evidence in favour of an enzyme-catalysed hydrolytic triggering is contained in a report that selective cleavage of spore cortex is the first detectable enzymic event in L-alanine-triggered germination of *Bacillus megaterium* KM spores (Johnstone & Ellar, 1982). We therefore decided to test the generality of this hypothesis by examining various aspects of the biochemistry of germination of *Bacillus fastidiosus* spores, which were reported to be triggered solely by uric acid (Aoki & Slepecky, 1973).

Materials and methods

Bacterial strain and preparation of spores

Bacillus fastidiosus N.C.I.B. 10016 was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland, U.K. It was grown on solid medium containing (w/v) 1% uric acid, 0.1% K_2HPO_4 and 3% agar (Difco), with the addition of 50 μM - MnCl_2 . In some experiments the medium contained 1% allantoin instead of uric acid. After 2–3 days of incubation at 30°C, the surface of the agar was scraped with a spatula, then washed with deionized water, and the spores were harvested by centrifugation (10000g for 5 min at 4°C). The pellet was washed with deionized water (4°C) until

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free from contamination by cells or debris. Finally, spores were suspended in cold deionized water and stored at 4°C until used. Normally, they were used within 5 days of harvest. During this time, there was no change in the germination rate of the spores.

Germination of the spores

Unless otherwise stated, spores were germinated at 37°C in 8.9mM-uric acid in 10mM-Tris, pH9.0 (the final pH of the solution before addition of the spores was adjusted with HCl to pH7.0). Spores were heat-activated in deionized water (70°C, 30min) before triggering of germination. Germination was measured by decrease in A_{600} of spore suspensions.

O₂ consumption

O₂ uptake by germinating spores was measured in an oxygen electrode (Rank Bros., Bottisham, Cambridge, U.K.) at 37°C.

Commitment to germination

Spores (2mg dry wt./ml) were incubated at 37°C in 8.9mM-uric acid dissolved in 10mM-Tris (final pH7.0). Samples (0.3ml) were diluted in tubes containing 3ml of 50mM-Tris/HCl buffer, pH8.5. After incubation at 37°C for 45min to allow committed spores to germinate, the A_{600} of the suspension was measured.

Ca²⁺ and dipicolinic acid contents of spores and release during germination

For measurements of Ca²⁺ content, spores were dried for 5h at 160°C and then ashed at 500°C for 2h. The ash was dissolved in 100 µl of conc. HCl and diluted to 0.1M-HCl. All samples contained 1% LaCl₃. Calcium was determined by using a Perkin-Elmer model 380 atomic-absorption spectrophotometer.

For dipicolinic acid measurements, spores were extracted with boiling propan-1-ol for 10min, and the samples were dried *in vacuo*. The residue was suspended in deionized water and, after centrifugation (25000g for 5min), the supernatant was assayed for dipicolinic acid as previously described (Scott & Ellar, 1978c).

For the determination of release of Ca²⁺ and dipicolinic acid during germination, spores (6mg dry wt./ml) were germinated, samples (1.5ml) were rapidly centrifuged in an Eppendorf Minifuge and the supernatants were assayed for calcium and dipicolinic acid as described above.

ATP content of spores and germinating spores

ATP pools were measured as previously described (Scott & Ellar, 1978a).

Preparation of cell-free extracts

Spores (30mg dry wt.) were suspended in 1.5ml of 50mM-Tris/HCl buffer, pH7.0, mixed with 8.0g of cold glass beads (0.10–0.11mm diam.) and broken in a Braun cell homogenizer for six periods of 30s with liquid-CO₂ cooling. This crude extract was used for the enzyme assays. In those experiments in which crude extracts were used to fractionate their components in order to locate the enzyme activities, the extracts were centrifuged at 25000g for 3min at 4°C to remove spore integuments, and the resulting supernatant was centrifuged at 105000g for 1h at 4°C to deposit the membrane fraction. Both pellets were washed twice with the above buffer. The supernatant from the 105000g centrifugation was used as the soluble fraction.

To prepare a vegetative-cell crude extract, cells from an overnight plate culture containing uric acid were transferred to 100ml of liquid medium containing 1% uric acid or 1% allantoin and incubated at 37°C and 200rev./min in a Gallenkamp orbital incubator until the A_{600} of the culture reached a value of 1.0. After centrifugation and washing twice with 50mM-Tris/HCl buffer, pH7.0, the cells were suspended in the same buffer (20mg dry wt./ml) and the crude extract was obtained as described above for spores.

Uricase and allantoinase activities

Uricase activity was measured by the method described by Mahler (1970). Allantoinase activity was determined by the differential analyses of glyoxylate derivatives (Vogels & Van der Drift, 1976). For both enzyme assays, 1 enzyme unit was defined as the amount of enzyme hydrolysing 1 µmol of uric acid or allantoin per min at 37°C under the conditions of the assay.

Dry-weight determinations

Samples were dried to a constant weight at 80°C on preweighed aluminium planchets.

Measurement of cortex hydrolysis during germination

Incorporation of radioactivity from NaB³H₄ by lysozyme-soluble cortical peptidoglycan reducing groups during germination was measured as previously described (Johnstone & Ellar, 1982).

Chemicals

NaB³H₄ (5Ci/mmol) was obtained from Amersham International, Amersham, Bucks., U.K. Uric acid and allantoin were from Sigma Chemical Co., St. Louis, MO, U.S.A. All the other reagents were of analytical grade.

Results

General features of germination

Previous work (Leadbetter & Holt, 1968; Kaltwasser, 1971; Bongaerts & Vogels, 1976) has shown that uric acid and allantoin are the only carbon sources capable of sustaining vegetative growth of *B. fastidiosus*. Scheme 1 shows the pathway by which catabolism of these compounds is believed to occur (Bongaerts & Vogels, 1976). As Aoki & Slepecky (1973) reported, we found that spores of *B. fastidiosus* only germinate in the presence of uric acid. In addition to the structurally related purines (xanthine, hypoxanthine, guanine, adenine and 2,8-dihydroxypurine), other intermediate metabolites of the catabolic pathway of uric acid (allantoin, allantoic acid, ureidoglycollic acid, glyoxylic acid, urea and NH_4Cl) were also tested as germinants or inhibitors of germination. None of these, even at concentrations of 20 mM, caused germination or affected uric acid-induced germination. L-Alanine, a common germinant for a variety of *Bacillus* spores, was also found to be inactive even in the presence of the inhibitor of alanine racemase *O*-carbamoyl-D-serine (5 mM), so that the production of inhibitory D-alanine is not an explanation for the failure of L-alanine to trigger germination in this organism.

It has been reported (Aoki & Slepecky, 1973) that, when *B. fastidiosus* spores are formed in the presence of Mn^{2+} , heat activation is required before germination can be triggered. In our strain, although heat activation is stimulatory, this requirement was not absolute. Thus spores formed in

medium without added Mn^{2+} germinated with or without heat activation, but the germination rate was higher in heat-activated spores. Spores harvested from Mn^{2+} -supplemented medium (50 μM) showed only a 10% decrease in A_{600} after 45 min of germination. Heat activation of these spores (70°C for 30 min) induced germination with a pattern similar to that of spores grown on non-supplemented medium and of non-heat-activated spores (results not shown).

Germination measured as the loss of A_{600} started 2 min after the addition of uric acid, and 50–60% of the initial A_{600} was lost in 30 min, with more than 90% of the spores becoming phase-dark after 45 min. There was no significant difference in the germination rates of spores harvested from media containing uric acid or allantoin. Germination showed a clear dependence on uric acid concentration (Fig. 1). Below 1 mM-uric acid, germination was negligible (less than 5% decrease in A_{600} after 45 min). A marked increase in the germination rate was observed when the concentration was raised from 1 to 6 mM, with the rate reaching a constant value from 6.5 to 8.9 mM. The apparent K_m for uric acid-induced germination was 4–5 mM. Between pH 6.7 and 7.8 the germination rate remained constant, with a sudden decrease above the latter pH. There was no germination above pH 8.5. Optimum germination took place between 30° and 37°C, the lower and the upper limits being 15° and 45°C respectively.

Consumption

O_2 uptake by germinating (heat-activated)

Scheme 1. Catabolic pathway of purine degradation and the proposed route of uric acid and allantoin utilization in *B. fastidiosus*

Details of the reaction catalysed by uricase and the associated catalase are shown in the insert.

Fig. 1. Influence of uric acid concentration on germination of *B. fastidiosus* spores

Spores were heat-activated and germinated at 37°C in the presence of different concentrations of uric acid. The germination rate is expressed as $100 \times A_{600}$ decrease/min and was calculated from the linear portion of the germination curve.

Fig. 2. O_2 consumption during germination of *B. fastidiosus* spores

Spores were heat-activated and germinated at 37°C in 8.9 mM-uric acid in 10 mM-Tris/HCl buffer, pH 7.0. ○, Control, no germinant (15 mg of spores/ml); ●, 5 mg of spores/ml plus germinant; △, 10 mg of spores/ml plus germinant; ▲, 15 mg of spores/ml plus germinant.

spores started immediately on addition of uric acid (Fig. 2). Control experiments showed that this was not due to the presence of contaminating vegetative cells or debris in the spore suspension.

Germination in the absence of O_2 was also studied. Anaerobic conditions were obtained after bubbling the uric acid solution and the spore suspension for 1 h with O_2 -free N_2 . Immediately before germination was initiated by mixing the spore suspension with the uric acid solution, $Na_2S_2O_4$ (2 mg/ml) was added. Only 10% of the initial A_{600} was lost in the next 45 min (Fig. 3). When aerobiosis was restored, 90% of the spore population became phase-dark after 45 min. Similar results were obtained without $Na_2S_2O_4$ addition.

ATP content and release of Ca^{2+} and dipicolinic acid during germination

B. fastidiosus spores were found to contain amounts of Ca^{2+} (25 mg/g dry wt.), dipicolinic acid (108 mg/g dry wt.) and ATP (2 pmol/mg dry wt.) that are typical of spores of a wide range of *Bacillus* species. On addition of uric acid, there was a rapid release of Ca^{2+} and dipicolinic acid without any apparent lag (Fig. 4). No increase in ATP occurred, however, until 2½ min into germination (Fig. 4).

Fig. 3. Effect of inhibitors on germination of *B. fastidiosus* spores

Heat-activated spores were germinated in 8.9 mM-uric acid in 10 mM-Tris/HCl buffer, pH 7.0, in the presence of different inhibitors: ○, control, no inhibitor; ●, 30 mM- NaN_3 ; △, 20 mM- NH_2OH ; ▲, 4 mM- $CuCl_2$; □, anaerobic conditions and 10 mM- $Na_2S_2O_4$.

Fig. 4. Release of Ca^{2+} and dipicolinic acid, synthesis of ATP and measurement of new cortex-reducing compounds during germination of heat-activated *B. fastidiosus* spores

○, Dipicolinic acid; ●, Ca^{2+} ; ■, ATP pools; □, radioactivity incorporated from NaB^3H_4 into lysozyme-soluble peptidoglycan. For details see the Materials and methods section.

Inhibitors

Uric acid-induced germination, measured as decrease in A_{600} , was inhibited by 30mM- NaN_3 , 10mM- $\text{Na}_2\text{S}_2\text{O}_4$, 4mM- CuCl_2 and 20mM- NH_2OH when these compounds were added together with uric acid (Fig. 4). If germination was first triggered by uric acid and these inhibitors were added subsequently, the A_{600} decrease was immediately halted (results not shown). Other potential inhibitors, such as 40mM-KCN, 40mM-diethyldithiocarbamic acid, 60mM-potassium oxonate, 40mM-NaF and 30mM-potassium arsenate, did not affect germination, as measured by A_{600} decrease, when added either before uric acid or while uric acid-triggered germination was in progress.

Commitment

As Fig. 5 shows, exposure of the spores to 8.9mM-uric acid for 5 min caused 50% of the spores to become committed to germinate. Controls were performed to check that the method used to prevent the initiation of germination (dilution at a very low uric acid concentration and an increase in the pH) prevented the germination of refractile spores while allowing the committed spores to continue the loss of A_{600} .

Fig. 5. Commitment to germination of *B. fastidiosus* spores. Heat-activated spores were exposed to 8.9mM-uric acid for different time periods, then diluted 10-fold in 50mM-Tris/HCl buffer, pH 8.5, and the A_{600} was measured after 45 min as described in the Materials and methods section.

Uricase and allantoinase activities

Cell-free extracts obtained from spores harvested from uric acid or allantoin medium contained both uricase and allantoinase activities (Table 1), showing that both enzyme activities are present in the dormant spore; 95% of the activity of these enzymes was located in the soluble fraction. The specific activities of these enzymes were much lower in dormant spores than in vegetative cells in the exponential phase of growth (Table 1). There was no difference between the uricase activity of heat-activated and non-heat-activated spores. Uricase activity in cell-free extracts of dormant spores

Table 1. *Uricase and allantoinase activities of dormant-spore and vegetative-cell extracts of B. fastidiosus*

The numbers in parentheses indicate the percentage of activity with respect to the total crude extract. Experimental details are given in the Materials and methods section.

	Sp. activity (units/g dry wt.)	
	Uricase	Allantoinase
Dormant spore		
Crude extract	26.15	11.52
Integument fraction	0 (0%)	0 (0%)
Membrane fraction	0.84 (3%)	0.13 (1%)
Soluble fraction	24.84 (95%)	10.94 (95%)
Vegetative cell		
Crude extract	905.15	652.18

Table 2. *Effect of inhibitors on uricase activity of dormant-spore extracts of B. fastidiosus*

Experimental details are given in the Materials and methods section.

	Inhibitor (mM)	Uricase activity (% inhibition)
KCN	1	4
	10	50
	50	85
Diethyldithiocarbamic acid	1	6
	10	26
	50	76
NaN ₃	1	7
	10	31
	50	64
NH ₂ OH	1	4
	10	31
	50	68
CuCl ₂	1	44
	10	72
	50	90
NaF	80	0
Na ₂ HAsO ₄	80	3
Na ₂ S ₂ O ₄	10	95

was inhibited by KCN, diethyldithiocarbamic acid, NaN₃, NH₂OH, Na₂S₂O₄, CuCl₂ and potassium oxonate and unaffected by NaF and Na₂HAsO₄ (Table 2). Oxonate was a particularly effective inhibitor, as previously reported by Vogels & Van der Drift (1970). The apparent *K_m* for this enzyme activity in dormant spores was 0.6–1.0 mM.

During the first 5 min of germination the specific activity of uricase increased slightly, from 26 to 31 units/mg dry wt. The presence of chloramphenicol (100 µg/ml) had no effect on this increase. This concentration of the antibiotic completely inhibited protein synthesis (results not shown). After this initial slight increase, uricase activity remained constant for at least the next 30 min of germination. No change was observed in the specific activity of allantoinase during the first 30 min of germination.

Measurement of cortex hydrolysis during germination

Cortex hydrolysis has been demonstrated to be the earliest detectable enzyme-catalysed event during the triggering of germination of *B. megaterium* KM spores (Johnstone & Ellar, 1982). It had previously been suggested that this hydrolysis could result in rapid rehydration of the dormant spore core and hence the onset of general spore metabolism (Ellar, 1978*a,b*). The appearance of cortical reducing groups was measured during germination of *B. fastidiosus* spores in order to investigate whether these spores respond to germinants in the same way as do those of *B. megaterium* KM. During the first 10 min of germination of heat-activated spores there was no detectable increase in the radioactivity incorporated from NaB³H₄ into spore cortex, however (Fig. 4).

Discussion

Bacillus fastidiosus has been shown to be a micro-organism with special requirements for vegetative growth, able to use uric acid or allantoin as a sole source of carbon and nitrogen (Leadbetter & Holt, 1968; Kaltwasser, 1971; Bongaerts & Vogels, 1976). From their experiments Aoki & Slepecky (1973) concluded that it was likely that spores of this micro-organism are even more fastidious in their germination requirements, and this dependence upon uric acid as the sole germinant has been reinforced by our results. It is evident that among a variety of compounds, including structurally related purines, intermediate metabolites of uric acid catabolism and compounds such as L-alanine, which is an effective germinant for other spores, only uric acid is capable of triggering *B. fastidiosus* spore germination. It was possible to

determine the minimum time for which the spores must be exposed to uric acid to enable them to continue germination after the triggering stimulus has been removed; 50% of the spores were found to be committed to germinate after 5 min exposure to uric acid.

Several studies of germination triggering in *B. megaterium* KM (Woese *et al.*, 1968; Scott & Ellar, 1978*a,b*; Scott *et al.*, 1978; Rossignol & Vary, 1978; Vary, 1978; Ellar, 1978*a,b*) by L-alanine and glucose have prompted the suggestion that triggering in this micro-organism involves either a biophysical event or initiation of a hydrolytic reaction that decreases or eliminates the rigid components of spore integuments. This hypothesis arose from experiments (Scott & Ellar, 1978*a,b*; Rossignol & Vary, 1978; Vary, 1978) that failed to detect any evidence for metabolism of either the germinant or endogenous spore intermediates during the period in which triggering occurs and demonstrated that a variety of metabolic and electron-transport inhibitors, including KCN, KF and anaerobiosis, all failed to prevent triggering of germination of *B. megaterium* spores. Support for this hypothesis comes from a report that selective cortex hydrolysis is the first detectable enzymic event in L-alanine-triggered germination of *B. megaterium* KM spores (Johnstone & Ellar, 1982).

Our results with *B. fastidiosus* strongly suggest that the above hypothesis cannot itself be extended to describe the mechanism of germination triggering in this micro-organism. In addition, our results suggest a role for uricase in the trigger mechanism. The following reasons support these views.

(i) No cortex hydrolysis was detected during the first 10 min of germination of heat-activated spores. Since 25% of the spore population germinated during this time, it is concluded that selective cortex hydrolysis does not constitute part of the trigger mechanism of germination in spores of *B. fastidiosus*.

(ii) Metabolic inhibitors (NaN₃ and anaerobiosis) inhibited *B. fastidiosus* spore triggering, in marked contrast with the results with other *Bacillus* spores (Scott & Ellar, 1978*a*; Vary, 1978), and also inhibited uricase activity.

(iii) In addition to these inhibitors, other compounds (CuCl₂ and NH₂OH) were effective germination inhibitors. All of these were inhibitors of uricase activity. Although both KCN and diethyl-dithiocarbamic acid inhibited uricase in spore and vegetative-cell extracts, these compounds did not inhibit germination. A similar result was obtained with the uricase inhibitor oxonate. The reason for this is not clear, but may be the result of the inability of these compounds to penetrate to the spore core. With KCN this seems unlikely, since this compound has been shown to be a potent

inhibitor of O₂ uptake during *B. megaterium* spore germination (Rossignol & Vary, 1978).

(iv) *B. fastidiosus* spores initiated O₂ uptake immediately on addition of uric acid, without any sign of the lag that is typical of other bacterial spores germinating in alanine or glucose (Wilkinson *et al.*, 1977; Rossignol & Vary, 1978). An apparent lag could be generated by decreasing the spore concentration in the oxygen-electrode chamber, which delayed the starting point of the O₂ uptake. If O₂ consumption were independent of uric acid degradation, an increase in the spore concentration would only produce an increase in the rate of O₂ uptake once it started, but the lag period would be unchanged.

(v) In contrast with the low K_m usually seen in other spores for germinants such as L-alanine, the apparent K_m for uric acid-induced germination of *B. fastidiosus* was high (4–5 mM). This may be compared with the K_m of uricase in cell-free extracts of dormant spores, which was 0.6–1.0 mM. The correlation between the apparent K_m values for germination and uricase activity may, however, be closer than this if the properties of spore membranes and integuments in any way restrict the entry of uric acid into the spore core, where the uricase is located.

(vi) Uric acid uptake experiments showed that after 5 min of germination in 8.9 mM-uric acid the amount of non-exchangeable uric acid taken up by the spores is 46 nmol/mg dry wt. (J. A. Salas & D. J. Ellar, unpublished work). If none of the uric acid that was accumulated had been metabolized, this would be the actual amount of uric acid in the spores. Fig. 2 shows that the total oxygen uptake by 1 mg of spores after 5 min of germination is approx. 16 nmol. If this uptake were solely the result of uric acid oxidation, the true value for O₂ uptake would be 32 nmol/mg of spores, since decomposition of the H₂O₂ formed during oxidation of each uric acid molecule will generate $\frac{1}{2}$ molecule of O₂ (Scheme 1). From stoichiometric considerations this would indicate an oxidation of 32 nmol of uric acid/mg of spores after 5 min, or approx. 70% of the non-exchangeable uric acid taken up at that time. The actual amount of uric acid that is oxidized is, however, likely to be less than this, since an undetermined proportion of the O₂ uptake will be the result of the re-activation of other oxidation reactions during the first minutes of germination.

(vii) Because only 50% of *B. fastidiosus* spores were found to be committed to germinate after 5 min (Fig. 5), these results suggest again that substantial metabolism of uric acid is necessary for germination triggering. This is in marked contrast with the very short commitment times recorded for L-alanine-triggered germination of other bacterial spores (Harrell & Halvorson, 1955; Hallmann &

Keynan, 1962; Watabe *et al.*, 1974; Stewart *et al.*, 1981) and the finding in at least three cases (Rossignol & Vary, 1978; Scott & Ellar, 1978a; Vary, 1978) that no detectable metabolism of the germinant occurs before commitment. In L-alanine-induced germination of *B. megaterium* KM it has been found that 10^3 – 10^4 molecules of the germinant need to be bound to each spore on average in order to obtain 50% commitment of the population (M. A. Koncewicz & D. J. Ellar, unpublished work). At 5 min of germination of *B. fastidiosus* spores, when 50% commitment has occurred, the results show that approx. 10^8 molecules of uric acid have been taken up by each spore. The difference of four or five orders of magnitude in the concentration of germinant required to cause commitment strongly suggests that triggering of *B. fastidiosus* spore germination is not brought about by the same biophysical or hydrolytic mechanism proposed for L-alanine- and glucose-triggered germination of other spore species.

The fact that the germination trigger for *B. fastidiosus* appears to be an enzyme that is essential for vegetative growth of this organism raises the possibility that these spores are merely quiescent rather than completely dormant and that this quiescence is induced when uric acid becomes limiting. However, as our experiments had shown, *B. fastidiosus* spores possess all the typical properties, such as absence of detectable metabolism, heat resistance, Ca^{2+} and dipicolinic acid content, low ATP content etc., that are characteristic of other bacterial spores classically considered as dormant. Furthermore, on germination, *B. fastidiosus* spores lose these spore characteristics and initiate ATP synthesis in a manner typical of dormant-spore germination. Other experiments in connection with the present study (results not shown) have revealed that these spores also display the same rapid chloramphenicol-resistant activation of the electron-transport chain that was reported for other *Bacillus* spores (Wilkinson *et al.*, 1977).

One interesting corollary of the use of uricase as the germination trigger in this micro-organism concerns the fate of the H_2O_2 produced in the reaction (Scheme 1). Since *B. fastidiosus* spores contain catalase (results not shown), the peroxide will yield not only $\frac{1}{2}$ mol of O_2 but also 1 mol of water for every mol of uric acid oxidized. Consequently, this oxidation of uric acid will lead to a small increase in the amount of water available for hydration of spore core constituents. If the absence of metabolism in dormant spores is the result of the relative dehydration of core enzymes or the immobilization of these enzymes in a dehydrated insoluble matrix of calcium dipicolinate, this fact

suggests a novel autocatalytic mechanism in *B. fastidiosus* by which the initiation of uricase activity might trigger the rehydration and hence re-activation of dormant-spore enzymes. Clearly, however, uricase, like the receptors for L-alanine and glucose in *B. megaterium* spores, is a spore protein that has escaped the dormancy-imposing mechanism. Since uricase appears to be a spore core enzyme, it will be interesting to investigate how this is achieved.

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