Isolation and Description of a Menaquinone Mutant from *Bacillus licheniformis*

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A menaquinone mutant (SG1) of *Bacillus licheniformis* has been isolated by selecting for colonies that are resistant to low levels of kanamycin (1.5 µg/ml) but sensitive to the same concentration of kanamycin in the presence of shikimate (25 µg/ml). The wild type (IU1) contained 0.38 ± 0.02 nmol of menaquinone-7 (MK-7) per mg (dry weight) of cells when grown ± shikimate, whereas SG1 had <0.01 nmol of MK-7 per mg (dry weight) of cells when grown without shikimate and 0.30 ± 0.02 nmol of MK-7 per mg (dry weight) of cells when grown in the presence of shikimate. SG1 had a generation time of 85 min, as compared to 24 min for IU1 grown ± shikimate. SG1 doubled with a generation time of 28 min when grown in the presence of shikimate. IU1 consumed O₂ at various rates depending on the stage of growth. A triphasic O₂ consumption curve with maxima at mid-exponential phase, the transition from exponential to stationary phase, and early stationary phase was found for IU1 ± shikimate and SG1 ± shikimate. SG1 grown without shikimate consumed O₂ at a low level (10 to 20% of IU1). Normal respiration could be restored to SG1 8.5 min after shikimate addition, whereas normal growth was not restored until 40 min after shikimate addition. Electron microscopic studies of SG1 and IU1 have indicated a morphological alteration in the mutant. SG1 is a dwarf cell as compared to IU1, when grown without shikimate. However, SG1 grown with shikimate became morphologically indistinguishable from IU1.

It has been established that vitamin K₂ is a component of the electron transport chain in many bacterial species. Other functions have been reported, such as involvement in pyrimidine synthesis in *Escherichia coli* (15), sphingolipid synthesis in *Bacteroides melaninogenicus* (13), and the ability to regulate sporulation in *Bacillus subtilis* (19). *Bacillus licheniformis* is an organism which has well-characterized membranes, contains menaquinone as the sole quinone, and possesses the ability to grow anaerobically. We undertook studies on this organism to determine whether menaquinone might play a role in determining membrane composition in addition to serving as an electron carrier. To this end we have isolated mutants of *B. licheniformis* deficient in menaquinone biosynthesis and report on the isolation, growth, O₂ consumption, and cell morphology of one such auxotroph.

(This work was taken in part from a dissertation submitted by S. R. G. to St. Louis University, St. Louis, Mo., 1976, in partial fulfillment of the requirements for the Ph.D. degree.)

**MATERIALS AND METHODS**

**Bacterial strains.** A wild-type *B. licheniformis* (IU1) was received from H. Gest. Our menaquinone-deficient mutant (SG1) was isolated as described below.

**Chemicals.** All organic solvents used were reagent grade, except for the spectral-grade hexane which was used for ultraviolet (UV) spectra and was purchased from EM Laboratories. The menaquinone homologues (MK-2 to MK-10) used as markers were the gift of Hoffmann-La Roche, Basel, Switzerland. Shikimate and kanamycin sulfate were purchased from Sigma Chemical Co., and ethyl methane sulfonate was purchased from Eastman Chemicals. The tryptose, beef extract, and tryptose blood agar base (TBAB) were purchased from Difco. Thin-layer chromatography (TLC) plates (0.25 mm) without fluorescent indicator were purchased from Brinkmann Instruments.

**Media.** The minimal salts medium (SMM) was that of Anagnostopoulos and Spizizen (1). The rich medium (TMG) contained, per liter: 10 g of tryptose, 5 g of NaCl, and 3 g of beef extract. Enough sterile glucose was added to give a final concentration of 0.25% glucose. The solid equivalent to TMG media is TBAB, which was purchased as premixed packs. Rich medium (TBABG) plates were made by adding 33 g of TBAB (Difco) to 1 liter of water. Glucose was added to a final concentration of 0.25% after autoclaving.

**Isolation of mutants.** IU1 was grown to 80 Klett units in 20 ml of TMG at 37 °C and harvested at 10,000
rpm for 8 min, and the cells were suspended in 2 ml of 50 mM tris(hydroxymethyl)aminomethane, pH 7.2. The cell suspension was mixed with 8 ml of 3% ethyl acetone, and turbidimetrically determined. This mixture was incubated with shaking at 37°C for 12 min, at which time 99.9% of the cells were killed. The cells were spun down and washed 3× with 20 ml of SMM, suspended in 2 ml of SMM, and then freeze-dried. The cells were then incubated at 37°C for 6 h. This treatment resulted in a 700-fold increase in the frequency of mutants resistant to 1.5 μg of kanamycin sulfate per ml.

Kanamycin-resistant colonies were replica plated onto TBABG, TBABG + shikimate (25 μg/ml), TBABG + kanamycin (1.5 μg/ml), and TBABG + kanamycin (1.5 μg/ml) + shikimate (25 μg/ml). Those colonies that grew on TBABG, TBABG + kanamycin, and TBABG + shikimate, but not grown on TBABG + kanamycin + shikimate, were selected for further study.

Growth. The rich medium supports growth both of wild-type (IU1) and men⁻ (SG1) mutants at 37°C. Since SG1 is blocked prior to shikimate in the menaquinone synthetic pathway, the rich medium must supply the other end products via the common aromatic pathway (aromatic amino acids and p-aminobenzoic acid). Cells were grown at 37°C in 10 to 30 ml of rich medium contained in 250-ml naphelometer flasks. Cell growth was determined turbidimetrically using a Klett-Summerson colorimeter equipped with a red filter. Both SG1 and IU1 were always pregrown in 20 ml of TMG media + shikimate (25 μg/ml) to 80 Klett units. The cells were harvested at 10,000 rpm for 8 min in a Sorvall centrifuge (10°C) and washed 3× with 10 ml of SMM. The washed cells were resuspended in 20 ml of SMM, and 0.5 ml was used to inoculate 30 ml of TMG without shikimate and 30 ml of TMG with shikimate (25 μg/ml). The cells were allowed to grow with shaking at 37°C.

Assays for menaquinone-7 (MK-7). IU1 and SG1 strains, which were pregrown in 20 ml of TMG + shikimate, were harvested, washed 3× with 10 ml of SMM, and suspended in 10 ml of SMM. This cell suspension (2 ml) was used to inoculate 500 ml of fresh TMG media in a 4-liter Erlenmeyer flask. A total of 1 liter of cells grown to 1 h into stationary phase was used for each menaquinone determination. The cells were harvested, washed with 20 ml of SMM, and then freeze-dried to a constant weight. The resulting powder was homogenized with 20 ml of acetone, and then an additional 80 ml of acetone was added and the extraction was allowed to proceed at 4°C for 6 h. The mixture was centrifuged at 10,000 rpm for 10 min, and the supernatant fluid was filtered to remove any remaining cell debris. The acetone was removed on a rotary evaporator, and the acetone-extractable lipids were dissolved in 10 ml of diethyl ether. The diethyl ether was removed under N₂, and the lipid was dissolved in 0.5 ml of methylene chloride and spotted on a TLC plate along with marker MK-7. In a developing system of isooctane-diethyl ether (100:30; vol/vol), the vitamin K₇ migrates to an R₃ of 0.52. The spot was visualized with a UV lamp, scraped from the plate, and extracted in diethyl ether.

The silica gel was removed by low-speed centrifugation, and the supernatant fluid was decanted and evaporated under N₂. The vitamin K₇ was dissolved in 3 ml of spectral-grade hexane, and the UV spectrum from 200 to 320 nm was obtained in a Beckman DB spectrophotometer. An ether extract of scrappings of the TLC plate above the solvent front was used as a blank. The extinction coefficient at the 248-nm absorption maximum was 1.9 × 10⁻³ liters/mole cm, and this value was used to quantitate MK-7 concentration. In control experiments in which radioactive vitamin K was added to the acetone extract and then taken through all the described steps, 95 to 97% of the radioactivity was recovered.

Oxygen consumption. Measurement of O₂ consumption was carried out at 37°C using a Clark-type oxygen electrode (Yellow Springs Instruments Co.).

Electron microscopy. IU1 and SG1 cells, which were pregrown and washed as described above, were used to inoculate fresh TMG media ± shikimate (25 μg/ml). The cells were grown at 37°C to mid-exponential growth stage (100 Klett units), harvested, and fixed at 4°C for 45 min in a solution of 30 mM glucose, 0.15 M sodium phosphate buffer, pH 7.3, and 0.45 mM CaCl₂, containing 5% glutaraldehyde. The fixed cells were washed four times with the glucose-phosphate-CaCl₂ solution, postfixed at 4°C for 45 min in the same solution containing 2% OsO₄, and washed once with the glucose-phosphate-CaCl₂ solution. The fixed cells were ethyl alcohol dehydrated, embedded in epon-araldite embedding resin, cut on an LKB-Huxley ultramicrotome, and double stained with uranylacetate and lead citrate. Micrographs were taken in a Philips 300 electron microscope at 60 kV.

RESULTS

Analysis of menaquinone from B. licheniformis. Salmon and Schmitt (16) have suggested MK-7 to be the homologue present in B. licheniformis, but their criterion was comigration with a standard on thin-layer plates developed in benzene or isooctane-diethyl ether (100:30, vol/vol), where one observes very poor resolution between MK-6, -7, and -8. Therefore, we extracted the UV-absorbing band that comigrates with MK-7 in isooctane-diethyl ether (100:30) at R₃ = 0.52 (Fig. 1) for analysis on paraffin-impregnated plates, which were developed in acetone-water (92:8, vol/vol) (7). This system gives clear resolution of the menaquinones, in particular MK-4 through MK-7. It can be clearly seen that vitamin K₇ from B. licheniformis comigrates with MK-7 at R₃ = 0.36.

Mass spectrometry. The mass spectra of both the bacterial and authentic MK-7 are shown in Fig. 2. Each spectrum shows the molecular ion at m/e 648 and a base peak at m/e 69 characteristic of menaquinones (5). Peaks at m/e 579, 511, 443, 375, 307, and 239 represent the loss of one terminal (69 mass units) and five internal (68 mass units) isoprene...
groups, respectively. The peak at m/e 225 corresponds to the chromylium ion formed from menaquinones by cyclization of the first isoprene unit and loss of the remainder of the side chain (6). The mass spectral data indicate further that the MK-7 from *B. licheniformis* has a normal isoprenyl side chain.

**Isolation of vitamin K₁ auxotrophs.** Aminoglycoside antibiotic resistance has been widely used to select for mutants blocked in some aspect of energy metabolism (8, 9, 11, 12, 14, 17-19). It has been suggested that these energy metabolism auxotrophs are unable to actively transport the aminoglycoside across their membrane (8).

We have sought menaquinone-deficient mutants among colonies resistant to 1.5 µg of kanamycin sulfate per ml. Only a small percentage of kanamycin-resistant mutants are menaquinone auxotrophs, and therefore further screening was necessary. If a cell is blocked in menaquinone synthesis and is kanamycin resistant because of this block, then one should be able to make that cell kanamycin sensitive by adding back an intermediate after the block, thereby allowing menaquinone synthesis and kanamycin transport. This is the rationale behind the replica-plating technique described in Materials and Methods.

Using this procedure, we have isolated several shikimate auxotrophs, and the phenotype of one such mutant is described in Table 1. In this and all other experiments to be described, IU1 and SG1 are always pregrown in the presence of shikimate (25 µg/ml) and then harvested and washed 3× with 10 ml of SMM. Samples of cell suspensions were used to inoculate two cultures, one containing TMG and the other containing

![TLC of menaquinones](image1)

**FIG. 1.** TLC of menaquinones. The cold acetone extract of 1 liter of cells grown into stationary phase (T1) was spotted on a TLC plate along with MK-7 marker and developed in isooctane-diethyl ether (100:30, vol/vol). The UV-absorbing band that comigrates with MK-7 was extracted from the plate with diethyl ether and rerun on a paraffin-impregnated plate in a developing system of acetone-water (92:8, vol/vol) with menaquinone markers (MK-2 to MK-7).

![Mass spectra](image2)

**FIG. 2.** Mass spectra of bacterial and authentic menaquinone. Mass spectra of menaquinone isolated by TLC of a cold acetone extract of *B. licheniformis* were run at 100 C, with an electron impact of 70 eV, using the direct probe.
TMG + shikimate (25 μg/ml), to give an initial cell density of 1 Klett unit. In the experiment described in Table 1, the cells were allowed to grow at 37 C to 100 Klett units and then were plated. The data in Table 1 show that: (i) IU1 is unaffected by having shikimate present in either the liquid or solid media; (ii) IU1 is sensitive to kanamycin (1.5 μg/ml); (iii) SG1 is resistant to kanamycin (1.5 μg/ml); (iv) SG1 is sensitive to kanamycin (1.5 μg/ml) in the presence of shikimate (25 μg/ml), but is not killed by shikimate (25 μg/ml) alone; (v) SG1, when grown with shikimate (25 μg/ml) in the liquid medium, behaves in a manner similar to IU1; (vi) there is ~1.8 times as many colony-forming units per Klett unit for SG1 grown without shikimate as compared to IU1 ± shikimate or SG1 + shikimate.

Results (i) through (v) suggest that SG1 is a menaquinone auxotroph blocked prior to shikimate in the synthetic pathway, whereas (vi) suggests that a morphological alteration also exists in SG1.

**MK-7 content of IU1 and SG1.** The concentration of MK-7 in IU1 and SG1 grown ± shikimate is presented in Table 2. IU1 grown with or without shikimate had ~0.38 nmol of vitamin K₇ per mg (dry weight) of cells. However, for SG1 grown without shikimate, there was no detectable menaquinone in an acetone extract of cells derived from a 1-liter culture harvested 1 h into stationary phase. There was no spot comigrating with MK-7 on TLC viewed with a UV lamp, and, when the region where MK-7 should run was scraped from the plate and eluted, no vitamin K₇ spectra were observed on scanning the UV range (200 to 320 nm) with a Beckman DB spectrometer. The limit of accurate detection using this technique is 0.01 optical density units at 249 nm because of trace UV-absorbing materials endemic to commercially available TLC plates. Therefore, we can assign a value of <0.01 nmol of MK-7 per mg (dry weight) of cells for SG1. When SG1 is grown with 25 μg of shikimate per ml present in the liquid medium, the MK-7 concentration is ~0.30 nmol of MK/mg of cells, indicating a block prior to shikimate in the menaquinone synthetic pathway of SG1.

**Growth and O₂ consumption.** Cultures for O₂ consumption and growth measurements were pregrown in the presence of shikimate, suspended in TMG ± shikimate as described in Materials and Methods, and then allowed to grow with shaking at 37 C (Fig. 3a and b).

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**Table 1. Growth of shikimate auxotroph on TBABG plate**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Growth on TBABG plates (CFU/Klett unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No addition</td>
</tr>
<tr>
<td>Wild type</td>
<td>510,000 (±40,000)</td>
</tr>
<tr>
<td>Wild type + shikimate</td>
<td>500,000 (±40,000)</td>
</tr>
<tr>
<td>Men⁻</td>
<td>920,000 (±30,000)</td>
</tr>
<tr>
<td>Men⁻ + shikimate</td>
<td>500,000 (±20,000)</td>
</tr>
</tbody>
</table>

* Wild type (IU1) and Men⁻ (SG1) were grown ± shikimate (25 μg/ml) to 100 Klett units in TMG media at 37 C. The cells were then plated on TBABG plates, with shikimate (25 μg/ml) and kanamycin (1.5 μg/ml) added in conjunction and separately, and the plates were allowed to incubate for 15 h at 37 C before colonies were counted. Values presented are the mean of triplicate plates. Numbers in parentheses represent the range of three determinations.

**Table 2. MK-7 in IU1 and SG1 grown ± shikimate**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>MK-7 (nmol/mg [dry wt] of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.38 ± 0.02 (5)</td>
</tr>
<tr>
<td>Wild type + shikimate (25 μg/ml)</td>
<td>0.37 ± 0.02 (3)</td>
</tr>
<tr>
<td>Men⁻</td>
<td>&lt;0.01 (4)</td>
</tr>
<tr>
<td>Men⁻ + shikimate (25 μg/ml)</td>
<td>0.30 ± 0.02 (3)</td>
</tr>
</tbody>
</table>

* One-liter batches of cells were grown at 37 C to 1 h into stationary phase. The cells are harvested, washed, freeze dried, and then acetone extracted. The vitamin K₇ was obtained as described in Materials and Methods. Wild type (IU1) ± shikimate and Men⁻ (SG1) + shikimate yield 150 ± 10 mg (dry weight) of cells per liter of culture grown to stationary phase. Men⁻ (SG1) yield 120 ± 10 mg (dry weight) of cells per liter of culture grown to stationary phase. Numbers in parentheses represent number of determinations.
The growth curve (Fig. 3a) for IU1 was unaffected by the presence of 25 μg of shikimate per ml. IU1 had a generation time of 24 min and grew to a final cell density of ~290 Klett units. SG1 grew just like IU1 for the first hour of shikimate starvation, and it was not until 1.5 h that we observed a deviation in growth (at this time, the cell density had increased from 1 to 6 Klett units). From this point to the onset of stationary phase, the mutant doubled, with a generation time of 85 min. SG1 reached a final cell density of ~120 Klett units; however, it should be remembered that the number of colony-forming units per Klett unit was 1.8 times greater in SG1 than in IU1. SG1 grown in the presence of shikimate had a growth curve resembling IU1, with a generation time of 28 min and a final cell density of ~290 Klett units.

The oxygen consumption curves for this experiment are shown in Fig. 3b. IU1 was unaffected by the presence of shikimate, and a triphasic O₂ consumption curve was observed, with maxima at the mid-exponential growth phase (1.7 h), the transition from exponential to stationary phase (3.7 h), and early stationary phase (7.5 h). During the 11-h growth period studied, IU1 varied from a maximum O₂ consumption of 0.52 μg-atom of O/min per Klett unit to a minimum of 0.24 μg-atom of O/min per Klett unit. SG1 O₂ consumption was similar to that of IU1 until 1.5 h of shikimate starvation in a manner similar to growth. At 5.5 h (60 Klett units), the O₂ consumption began to slowly decrease towards a constant value of 0.05 μg-atom of O/min per Klett unit, a value which is 10 to 20% of the values observed for IU1 over the same growth period. SG1 grown in the presence of shikimate had an O₂ consumption curve resembling the triphasic curve of IU1.

To observe the kinetics of recovery from menaquinone starvation, SG1 was pregrown in TMG + shikimate (25 μg/ml) and then harvested and washed; enough cells were added to 30 ml of fresh TMG to give a Klett reading of 1 Klett unit. These cells, starved for shikimate, were allowed to grow to 20 Klett units, at which time the culture was split and one-half received enough shikimate in 25 μl of water to give a final concentration of 25 μg/ml, whereas the other half received only 25 μl of sterile water. The results shown in Fig. 4 indicate that the portion of the culture receiving only water continued growing unaffected, with a generation time of 85 min. The portion receiving shikimate was unaffected for the first 10 min, showed increased growth at 20 min, and began its maximal growth rate (generation time, 28 min) at 40 min. In a parallel study, IU1 was unaffected by shikimate addition (data not shown).

When the same cells that had reacted 20 Klett units during shikimate starvation were placed in an O₂ electrode chamber, they consumed O₂ at a steady low level (0.12 μg-atom of O/min per Klett unit). This low rate of O₂ consumption was only slightly affected by addition of 5 μl of sterile water. But addition of enough shikimate in 5 μl of water to give a final concentration of 25 μg/ml gave a large and rapid

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**Fig. 3.** (a) Growth curves for wild type and Men⁻ mutant of B. licheniformis. Men⁻ (SG1) and wild type (IU1) were pregrown in 20 ml of TMG + shikimate (25 μg/ml) to 80 Klett units and then harvested, washed 3× with SMM (10 ml), and resuspended in 20 ml of SMM. Enough cells (0.5 ml) were added to 30 ml of TMG ± shikimate (25 μg/ml) to give an initial Klett reading of 1. Cells were allowed to grow at 37 °C with readings taken on a Klett-Summer-son colorimeter every 0.5 h. (b) O₂ consumption of wild type (IU1) and men⁻ (SG1) mutants of B. licheniformis as a function of time.
effect (Fig. 4). Increased O₂ consumption was seen 2 min after shikimate addition, and a maximal level of O₂ consumption (0.34 µg-atom of O/min per Klett unit) was reached in 8.5 min. Therefore, maximal respiration was resumed 8.5 min after shikimate addition, whereas normal growth was not resumed until 40 min. In a parallel study, O₂ consumption of IU1 was found to be unaffected by shikimate (data not shown).

**Electron microscopy.** Electron microscopic studies of SG1 and IU1 ± shikimate revealed that the SG1 mutant is a dwarf cell with dimensions of 1.4 to 1.6 µm (length) and ~0.5 µm (width) (Fig. 5). IU1 ± shikimate and SG1 ± shikimate are large cells with dimensions of 2.5 to 2.9 µm (length) and 0.6 µm (width). These results, giving the relative sizes of cells harvested at 100 Klett units, are consistent with our results shown in Table 1 which indicated that there were 1.8 times as many colony-forming units per Klett unit for SG1 as for IU1 ± shikimate or SG1 ± shikimate.

**DISCUSSION**

The first menaquinone auxotrophs in *B. licheniformis* have been isolated by selecting for colonies resistant to low levels of kanamycin (1.5 µg/ml) but sensitive to kanamycin (1.5 µg/ml) in the presence of shikimate (25 µg/ml). This is a technique which should have general application for the isolation of auxotrophs blocked elsewhere in the menaquinone biosynthetic pathway, simply by varying the intermediate used in replica plating.

Mass spectral analysis has indicated that MK-7 is the vitamin K₉ homologue present in *B. licheniformis*. The concentration of MK-7 in IU1 *B. licheniformis* was found to be 0.38 ± 0.02 nmol/mg (dry weight) of cells, and this MK-7 concentration was unaffected by the presence of 25 µg of shikimate per ml. SG1 was found to contain <0.01 nmol of MK-7 per mg (dry weight) of cells when grown without shikimate and 0.30 ± 0.02 nmol of MK-7 per mg (dry weight) of cells when grown in the presence of 25 µg of shikimate per ml.

O₂ consumption for IU1 followed a triphasic curve, with maxima at mid-exponential growth phase, the transition from exponential to stationary phase, and early stationary phase. Similarly, Farrand and Taber (9) found two maxima for *B. subtilis* in mid-exponential and transition growth stages, corresponding to our first two maxima; they did not report on the consumption of stationary-phase cultures. SG1 when starved for shikimate has less than 3% of the IU1 level of vitamin K₉, but still consumes O₂ at

![Fig. 4. Restoration of normal O₂ consumption and growth after addition of shikimate to SG1. Men⁻ (SG1) was pregrown in 20 ml of TMG + shikimate (25 µg/ml). The cells were harvested, washed, and reinoculated into 30 ml of fresh TMG without shikimate as described in Materials and Methods to an initial Klett reading of 1. The cells were allowed to grow to 20 Klett units in the absence of shikimate, and then the culture was split, with one-half receiving 25 µl of enough shikimate to give a final concentration of 25 µg/ml and the other half receiving 25 µl of sterile water. Klett readings were taken every 10 min. O₂ consumption of men⁻ (SG1) grown to 20 Klett units without shikimate was followed polarographically before and after addition of 5 µl of enough shikimate to give a final concentration of 25 µg/ml. A control received 5 µl of sterile water.](http://jb.asm.org/)

10 to 20% of the IU1 rate. There are two explanations for this residual O₂ consumption: (i) *B. licheniformis* can carry on a low level of respiration independent of menaquinone and/or (ii) trace amounts of MK-7 can support this low level of O₂ consumption. In support of the first explanation, a vitamin K-independent oxidase, which has an artificial bypass from reduced nicotinamide adenine dinucleotide dehydrogenase to the terminal oxidase, has already been suggested (3) to explain residual reduced nicotinamide adenine dinucleotide oxidase activity. This activity is not destroyed by irradiation or lipid extraction techniques, which reduce menaquinone in *Mycobacterium phlei* (3) and *Micrococcus lysodeikticus* (10) to undetectable levels. In addition, succinoxidase has been found not to have a direct quinone requirement in several bacterial species (2, 9). Therefore, it is reasonable that our SG1 mutant might carry on a low level of respiration in the absence of menaquinone, although a contribution from minute levels of MK-7 cannot be ruled out at present.

Of great interest was the fact that there is a
lag period of about 32 min between the times for restoration of normal respiration and normal growth after shikimate addition to SG1. Clearly, restoration of normal respiration is not sufficient to restore the IU1 phenotype to SG1. Some other changes either directly or indirectly related to the menaquinone mutation must occur during this lag period before normal growth can be restored.

Finally, we have described a morphological alteration related to the menaquinone mutation. SG1 is a dwarf cell as compared to IU1 ± shikimate and SG1 + shikimate. The question of whether this morphological alteration is a direct effect of the menaquinone mutation or a secondary effect due to altered respiration or growth is currently under investigation by taking advantage of the fact that B. licheniformis is a facultative anaerobe.

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LITERATURE CITED


