Cloning of an *Erwinia herbicola* Gene Necessary for Gluconic Acid Production and Enhanced Mineral Phosphate Solubilization in *Escherichia coli* HB101: Nucleotide Sequence and Probable Involvement in Biosynthesis of the Coenzyme Pyrroloquinoline Quinone

SHIH-TUNG LIU,^{1*} LAN-YING LEE,¹ CHIN-YING TAI,¹ CHIEN-HUI HUNG,¹ YU-SUN CHANG,¹
JAMES H. WOLFRAM,² ROBERT ROGERS,² AND ALAN H. GOLDSTEIN^{3*}

Department of Microbiology and Immunology, Chang-Gung Medical College, Kwei-Shan Taoyuan, Taiwan¹;
Biotechnology Group, Idaho National Engineering Laboratory, Idaho Falls, Idaho 83415²; and
Department of Biology, California State University at Los Angeles,
5151 State University Drive, Los Angeles, California 90032³

Received 28 April 1992/Accepted 16 July 1992

Escherichia coli is capable of synthesizing the apo-glucose dehydrogenase enzyme (GDH) but not the cofactor pyrroloquinoline quinone (PQQ), which is essential for formation of the holoenzyme. Therefore, in the absence of exogenous PQQ, *E. coli* does not produce gluconic acid. Evidence is presented to show that the expression of an *Erwinia herbicola* gene in *E. coli* HB101(pMCG898) resulted in the production of gluconic acid, which, in turn, implied PQQ biosynthesis. Transposon mutagenesis showed that the essential gene or locus was within a 1.8-kb region of a 4.5-kb insert of the plasmid pMCG898. This 1.8-kb region contained only one apparent open reading frame. In this paper, we present the nucleotide sequence of this open reading frame, a 1,134-bp DNA fragment coding for a protein with an M_r of 42,160. The deduced sequence of this protein had a high degree of homology with that of gene III (M_r , 43,600) of a PQQ synthase gene complex from *Acinetobacter calcoaceticus* previously identified by Goosen et al. (J. Bacteriol. 171:447–455, 1989). In minicell analysis, pMCG898 encoded a protein with an M_r of 41,000. These data indicate that *E. coli* HB101(pMCG898) produced the GDH-PQQ holoenzyme, which, in turn, catalyzed the oxidation of glucose to gluconic acid in the periplasmic space. As a result of the gluconic acid production, *E. coli* HB101(pMCG898) showed an enhanced mineral phosphate-solubilizing phenotype due to acid dissolution of the hydroxyapatite substrate.

Quinoproteins play a major role in the regulation of bioenergetic processes in many gram-negative bacteria, including *Erwinia* spp. (2). For many species of *Erwinia*, the nonphosphorylating oxidation pathway is the primary mechanism for aldose sugar utilization (2). The quinoprotein glucose dehydrogenase (GDH) controls the unique step in this metabolic pathway. As such, GDH plays a direct role in the generation of the transmembrane proton motive force via the oxidation of aldose sugars (5). It is now generally accepted that, in gram-negative bacteria, the membrane-bound quinoprotein GDH is present on the periplasmic side of the cytoplasmic membrane. GDH is a member of the largest group of quinoproteins, those that use the cofactor 2,7,9-tricarboxyl-1H-pyrrolo[2,3-f]quinoline-4,5-dione (PQQ) (5).

Escherichia coli does not synthesize PQQ, but it does synthesize apo-GDH and is therefore dependent on uptake of PQQ from the environment or culture medium (9). Binding of the cofactor is presumably simplified by the location of the apoenzyme on the outer face of the cytoplasmic membrane. The GDH holoenzyme may be formed in *E. coli* when functional genes for PQQ biosynthesis are introduced. This system has been elegantly exploited by Goosen et al. (9) in order to identify and isolate PQQ synthase genes from *Acinetobacter calcoaceticus* on the basis of their expression on plasmids cloned into *E. coli*. The expression of PQQ

synthase genes in *E. coli* resulted in GDH activity in the absence of exogenous PQQ. We have used a slight modification of this system to identify a PQQ synthase gene from *Erwinia herbicola* EHO10 (18). Our experimental approach included a unique phenotypic screening system based on our interest in elucidation of the metabolic basis for the mineral phosphate solubilization (Mps^+) phenotype in gram-negative bacteria.

Poorly soluble mineral phosphates such as hydroxyapatite (HAP) are dissolved via acidification (7, 8). The bacterial Mps^+ phenotype may be assayed via the addition of HAP or other poorly soluble phosphates to the culture medium. On agar plates, solubilization may be visualized directly by clearing zones, whereas in liquid culture the level of P_i may be determined analytically. As expected, bacteria that produce large amounts of gluconic acid, such as *E. herbicola*, also show high levels of mineral phosphate solubilization, whereas *E. coli* strains are usually poor solubilizers. Our strategy has been to clone genes from *E. herbicola* into *E. coli* in order to study the encoded gene products involved in the Mps^+ phenotype. It was presumed that genes responsible for the Mps^+ phenotype and genes controlling organic acid production and/or secretion would be identical or closely related (7, 8).

In this paper, we report the construction of a plasmid containing a functional mineral phosphate-solubilizing gene as defined by the HAP-solubilizing phenotype. Data that strongly suggest that the metabolic function of the protein encoded by this gene is the biosynthesis of PQQ, which, in

* Corresponding authors.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Characteristics	Source or reference
Strains		
<i>Erwinia herbicola</i> EHO10	Wild type	18
<i>Escherichia coli</i> HB101	F ⁻ <i>hsdS20</i> (r _B ⁻ m _B ⁻) <i>supE44 recA13 ara-14 proA2 rpsL20</i> (Str ^r) <i>xyl-5 mtl-q1 galK2 lacY1 λ</i> ⁻	4
MG1063	<i>recA</i> F ⁺ ::Tn1000	10
P678-54	F ⁻ <i>thr leu thi supE lacY fhuA gal mal xyl ara mtl min</i>	C. I. Kado laboratory, University of California at Davis
Eco898	HB101::Tn5	S.-T. Liu laboratory
Plasmids		
pSL775	A cloning vector using pigment genes of <i>E. herbicola</i> as a selection marker; 7.0 kb	14
pMCG898	A deletion derivative of pIMPS1 containing a complete PQQ synthase gene	This study

turn, results in the assembly of the GDH holoenzyme, are presented. We report the complete nucleotide sequence of this gene and show that the deduced amino acid sequence shows a high degree of homology to that of gene III of the four-gene PQQ synthase complex from *A. calcoaceticus* identified by Goosen et al. (9).

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* was cultured in Luria-Bertani (LB) broth (16) at 37°C. *E. herbicola* was cultured in the same medium at 28°C. The antibiotics used for selection were ampicillin (Ap) (100 µg/ml) and kanamycin (50 µg/ml).

Subcloning. Plasmids were screened according to the method of Kado and Liu (12). Restriction enzymes, T4 DNA ligase, and *E. coli* DNA polymerase I (Klenow fragment) were purchased from Boehringer Mannheim Biologicals and were used according to the methods described by Maniatis et al. (16). *Hind*III fragments of the insert of cosmid pIMPS1 (8) were subcloned into the *Hind*III site of the pBR322-derived vector pSL775 (14). The clone pB2 (27.6 kb) containing *Mps*⁺ activity was selected on M9-HAP agar. This plasmid was further deleted by *Bam*HI (pB8, 13.3 kb), digested by *Ban*II, and religated, which resulted in pMCG898 (Fig. 1), which retained the ability to confer the HAP-solubilizing phenotype to recipient *E. coli*.

Tn1000 mutagenesis. Tn1000 insertional mutagenesis (gamma-delta) was carried out according to the method of Guyer (10). Plasmid pMCG898 was transformed into MG1063. pMCG898::Tn1000 insertional mutants were transferred into Eco890 by conjugation. The resulting transconjugants were cultured in LB broth with kanamycin and then plated on LB agar containing Ap and kanamycin. The locations and orientations of the Tn1000 insertions were determined by restriction mapping with *Bam*HI.

Preparation of minicells and analysis of plasmid-encoded proteins. The plasmids pMCG898 and pBR322 were transformed into the minicell-producing strain P678-54 (17). Cultures were grown overnight in LB-Ap broth at 37°C. Minicells were purified by sucrose density-gradient centrifugation, and plasmid-encoded proteins were labelled with 45 µCi of L-[³⁵S]methionine (1,020 Ci/mmol; Amersham Corp.) according to the method of Meagher et al. (17). The minicells were

lysed, and the proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (13).

DNA sequencing. Supercoiled plasmids were sequenced

FIG. 1. Subcloning strategy and map of pMCG898 carrying a putative *E. herbicola* PQQ synthase gene. This gene was originally cloned as part of a 55-kb insert in the cosmid vector pHC79 (8).

according to the chain termination method of Sanger et al. (19). DNA was sequenced with the gamma and the delta primers according to the method of Liu et al. (15). Gaps were filled by primers synthesized after results were obtained from the gamma-delta sequencing (primer walking). DNA from both strands was sequenced. The primers were synthesized with a Cruachem PS250 DNA synthesizer. Sequence analysis, translation, and comparisons with previously identified PQQ synthase genes were done by using the Intelligenetics Suite program (11).

Changes in medium pH during bacterial growth. Bacteria were cultured overnight in LB-Ap. Cells were harvested by centrifugation and were washed three times with LB-Ap. Cells (from a 5-ml culture) were inoculated into 250 ml of M9WP culture medium. Media (20 ml) were sampled every 6 h, the cells were centrifuged, and the supernatants were passed through Millipore filters. The pH values were determined, and 100 μ l of the supernatants was spotted on M9WP-HAP medium for the determination of HAP solubilization. M9WP-HAP is a modified M9 (16) minimal medium, with soluble P_i replaced by HAP as previously described (8), 1% glucose as a carbon source, and appropriate amino acids and antibiotics (8). M9WP is the same medium without added HAP.

Mineral phosphate solubilization and determination of organic acids during bacterial growth. Liquid medium (GMS medium) consisted of (for 1 liter) 10 g of glucose, 2 g of $(NH_4)_2SO_4$, 0.3 g of $MgCl_2 \cdot 6H_2O$, 0.001 g of $MnCl_2 \cdot 4H_2O$, 0.0006 g of $FeSO_4 \cdot 7H_2O$, and 0.0006 g of $NaMoO_4$. Amino acids were added to 20 μ g/ml, and Ap was added to 50 μ g/ml. Tricalcium phosphate was present at 20 mg/ml. A 50-ml volume of media was inoculated with 5 ml of 48-h-old (stationary phase) liquid culture of the various bacteria. After being inoculated, flasks were placed on a shaker and grown at either 30°C (*E. herbicola*) or 37°C (*E. coli*). One-milliliter samples were taken daily. The concentration of soluble P_i and plate counts were determined from these samples. Organic acids were separated by high-performance liquid chromatography with a Beckman Interaction Ion 300 column. Detection was via A_{220} . The identification of unknown organic acids was based on comparison of the elution times of unknowns with the elution times of multiple standards. The detection limit for gluconic acid was 10 ppm.

Nucleotide sequence accession number. The GenBank accession number of the nucleotide sequence of the PQQ synthase gene of *E. herbicola* EHO10 is M94448.

RESULTS AND DISCUSSION

The screening strategy for the isolation of clones was based on the fact that, in plate assays, HB101 cannot visibly solubilize poorly soluble mineral phosphates such as HAP and tricalcium phosphate, whereas *E. herbicola* can (8). Plasmid pMCG898 (insert size, 4.5 kb) (Fig. 1) was originally derived from the cosmid clone pIMPS1, which contained a 55-kb insert of *E. herbicola* DNA. This cosmid was identified by Goldstein and Liu by its ability to confer the HAP-solubilizing phenotype to HB101 (8).

As shown in Fig. 1 and 2, a combination of subcloning and transposon mutagenesis data indicated that a 1.8-kb region of *E. herbicola* DNA was sufficient to confer the GDH⁺ Mps⁺ phenotype to *E. coli*. On the basis of selection with M9-HAP agar with appropriate supplements, we screened a total of 113 insertion mutants that each had a copy of pECO898::Tn1000. Twenty-five were Mps⁻, and the rest were Mps⁺. These 25 mutants had insertions in nine loca-

FIG. 2. Transposon Tn1000 mutagenic analysis of the region of pMCG898 necessary for gluconic acid production and the Mps⁺ phenotype. The nine mutants shown in this figure were contained within an approximately 1.8-kb region. These insertions simultaneously eliminated gluconic acid production and the Mps⁺ phenotype. Insertions outside this region did not affect either the GDH⁺ or the Mps⁺ phenotype. Insertions in the gamma-delta orientation (○) and the delta-gamma orientation (●) are indicated.

tions that covered an approximately 1.8-kb segment, as shown in Fig. 2. Data based on the size of the insert region, the restriction map, and the sequencing results strongly indicated that all Mps⁻ insertions were located in one gene. We did not find any Mps⁻ mutants that had insertions outside this 1.8-kb region.

Data shown in Fig. 3 indicate that one or two proteins were encoded by genes carried on pMCG898. Plasmid pSL775 was originally derived from pBR322 (14). In pMCG898, most of the pSL775-specific sequence was deleted so that pBR322 was used as the control in minicell

FIG. 3. SDS-PAGE of [³⁵S]methionine-labelled proteins encoded by pMCG898 in minicells. Proteins encoded by pBR322 (lane A) and synthesis directed by pMCG898 (lane B) are shown. A Klenow fragment repair-induced base insertion at the *Bam*HI site of pMCG898 caused the loss of the 33-kDa protein (lane C), while a similar insertion at the *Ban*II site did not affect the synthesis of this protein (lane D). The indicated proteins with M_r s of 41,000 and 32,000 are apparently encoded by the 4.5-kb insert.

```

1   ATGACTGACC   ACGCGCCAC   GGTGACCCA   CCGCTCTGGC   TGCTGGCGGA   GCTGACCTAC
60  CCGTGCCCGC   TGCAGTGCCC   CTACTGCTCC   AACCCACTCG   ATTTGCGCCG   CCAAGAAAAA
120 GAGCTGAGTA   CCGAGCAGTG   GATCGAGGTA   TTTCGCCAGG   CACGCGCGAT   GGGCAGCGTG
180 CAGTTGGGCT   TCTCGGCGGG   CGAGCCGCTG   GTGCGTAAAG   ATCTCCCCGA   GCTGATCGCC
240 GCCGCGCGCG   ATCTCGGCTT   TTACACCAAC   CTGATCACCT   CCGGCATCGG   CCTGACCGAG
300 AAGAAAGCTG   AGACCTTCGC   CGATGCCGGG   CTGGACCATA   TTCAGATCAG   CTTTCAGGCC
360 AGCGACGAGA   CGTTAAACGC   GGCCTCTGGC   GGCTCTGCCA   AAGCATTTCG   GCAGAGCTG
420 ACAATGCGCA   AAGCGGTTAA   GGCCTCTGGC   TATCCGATGG   TGCTCAACTT   CGTGCTGCAT
480 CGCCATAATA   TCGATCAGAT   TGACCGTATC   ATCGAGCTGG   CGATCCAGCT   GGATGCCGAT
540 GACGTAGAGC   TGGCAACCTG   CCAATTCTAC   GGCTGGGCGC   ACCTGAATCG   CGAAGGCGTG
600 CTTCCACACC   GCGAACAGAT   CGCCAAAGCA   GAGAGCGTGG   TCCAGCGCTA   CCGCGAACGC
660 ATGGCCGTCG   ACGGCAAGCT   GGTAACTCTG   CTGTTTGTCG   CGCCGGACTA   CTATGAGGAG
720 CGGCCAAAGG   GCTGTATGGG   CGGTTGGGGA   GCCATTTTCA   TGAGCGTGAC   GCGGAAAGGA
780 ATGGCCCTGC   CCTGCCACAG   TGCCCGTCAG   CTGCCGCTGA   AGTTTCCGTC   GGTGCTGGAA
840 CACGATCTCG   AGCATATCTG   GTATCACTCC   TTCGGCTTTA   ACCGTTACCG   CGGCATGCGG
900 TGGATGCGGG   AGCCGTGCCG   ATCTTCCCGG   GATGATGCGG   ACCCGGTGTG   TGCTAARTCG
960 TGCCAGGCTT   TCATGCTCAC   CGCGGACGCT   GATGATGCGG   ACCCGGTGTG   TGCTAARTCG
1020 CCCCATCACG   GCACCATTTT   GGCGGCGCGA   GAGCGGCGAA   ACCGACCCCA   GCTCGGCTAT
1080 GATCAGCTGC   GTTTCGCGAA   TCAGGCGAAC   TCCCGGCTGT   TTTCAAAAGG   CTAA

```

FIG. 4. DNA sequence of the open reading frame (starting at +1) presumed to be coding for the *mps* gene contained in the 1.8-kb region identified by transposon mutagenesis as essential for gluconic acid production by *E. coli* HB101(pMCG898).

analyses. Three proteins with apparent M_r s of 41,000, 33,000, and 32,000 were synthesized by pMCG898 but not by pBR322. When a base insertion was generated at the *Bam*HI site of pMCG898 by Klenow repair, the protein with an M_r of 33,000 was no longer synthesized (Fig. 3, lane C). However, a base addition at the *Ban*II site did not eliminate the synthesis of this protein (Fig. 3, lane D), suggesting that the proteins with M_r s of 41,000 and 32,000 were encoded by the *E. herbicola* DNA insert, while the protein with an M_r of 33,000 was encoded by the fusion sequence of the vector and the insert. As discussed below, the open reading frame identified within the 1.8-kb region of pMCG898 essential for gluconic acid production encoded a protein with a deduced M_r of 42,160, which compares favorably with the protein with an M_r of 41,000 seen in minicell analysis.

We obtained 1,960 bp of sequence covering the entire 1.8-kb region identified by *Tn1000* mutagenesis as essential for the GDH⁺ Mps⁺ phenotype. Figure 4 shows the complete sequence of the only significant open reading frame contained within this fragment. This open reading frame is 1,134-bp long and codes for a protein with a deduced M_r of 42,160. Figure 5 compares the deduced amino acid sequence of the putative PQQ synthase gene with that of gene III of a multigene complex involved in the synthesis of PQQ in *A. calcoaceticus* (9). It may be seen that, overall, there is 60% identity between the *E. herbicola* gene and gene III of *A. calcoaceticus*.

At the end of 24 h in liquid culture, *E. herbicola* had lowered the pH of the media to 3.5. HB101(pMCG898) lowered the pH to approximately 4.0 by the end of the same time period. HB101(pMCG898-50) (a pMCG898:*Tn1000* mutant with an insertion outside the 1.8-kb Mps region) gave a similar phenotype. HB101(pBR322) was not capable of lowering the pH of the media below 5.2 under the same conditions. HB101(pMCG898-51) (*Tn1000* insertion within the 1.8-kb Mps region) was also unable to lower the pH below 5.2 (pH data not shown).

Table 2 shows the amount of soluble P_i released from tricalcium phosphate medium by the various bacteria. It may be seen that, by day 7, HB101(pMCG898) had solubilized approximately 81% as much tricalcium phosphate as *E. herbicola*. HB101 and HB101(pBR322) were capable of

solubilizing only 24 and 32% of the tricalcium phosphate solubilized by *E. herbicola* during the same time period. Each value represents the average of two separate experiments. Numbers of CFUs were similar for the various bacterial strains (not shown).

From Table 2, it may also be seen that gluconic acid was detectable in the media of the *E. herbicola* and the HB101(pMCG898) cells but not in the media of the HB101 or the HB101(pBR322) cells. *E. herbicola* had carried out most of its mineral phase solubilization by day 3. Likewise, the

```

MTDHAPTVNPPLWLLAELTYRCPLQCPYCSNPLDFAAQEKELSTEQWIE VF
MTEGVGLPLWLLAELTYRCPLQCPYCSNPLDYAQHKNELTQEWDFVF

RQARAMGVSQVQLGFSAGEPLVRK DLPELI AAA RDL G FYTNLTSG I GLTEKLLQ
DQARQMGAVQLGFSGGELPLVRQDLQLELAHA HQQGFYTNLTSGMGLTEQRIA

TFAD AGLDHIQ I SFQASDETLNA ALAGSAK AFQQKLT MAKAVKALGYPMVLN
DLKQ AGLDHIQVSFQASDPVNDALAGSKH AFEQKYEMCRLVKKYDYPMVLN

FVLHRHN IDQ IDR I I ELAIQLDADDVELATCQFYGWAHLNREGLL FTREQ IAKA
FV IHRHN IDQ IEQ I I ELCLNELNADTVELA ICQFYGWAF LNRQGLLPTQEQLTR

ESVQRYRERMAVDGKLANIL LFVTFDYYEERPKGCMGGWGA IFMSVTPEG
ER I TNEYREKL KAQNHPCKL FVVPDY YEERPKAQMNGWGK IFFT VAPOG

MALPCHSARQLPVKFPVSL EHDLEHIWYHSFGFNRYRGNAWMPEPCRSCLP
MALPCHAAARQLP IS FPNVREHKLSDI WYKSTGFNHFGRDAWMPEGCRSCP

EKEKDFGGSCCOAF MLTGADNADNPVCAKS PHHGT IL AAREANRTQLGID
DKDRDFGGCRQAYMLTGDAANADNPVCGKS PYHQMQEQAQAEASQLVAPLQN

QLRFNRQANSRLFYKG
LVFRNSRNSKS LSATQNPVHTITDI

```

FIG. 5. Alignment of the deduced amino acid sequences of *E. herbicola mps* gene (upper) and gene III of the multigene complex involved in the synthesis of PQQ in *A. calcoaceticus*. Regions of identity are indicated by connecting dots.

TABLE 2. Production of gluconic acid and release of P_i from tricalcium phosphate by the different bacterial strains in liquid culture^a

Strain	Days in culture	P _i released (μg/ml)	Gluconic acid (integrated area units)
EHO10	3	350	8.65
HB101(pMCG898)	7	300	4.12
HB101	7	88	ND ^b
HB101(pBR322)	7	115	ND
Blank ^c	7	8	ND

^a Data are the averages of two separate experiments.^b ND, not detected.^c No bacteria present.

gluconic acid peak was detected at day 3. The appearance of the gluconic acid peak in HB101(pMCG898) was delayed until later in the experiment and peaked at day 7, as did maximum mineral phosphate solubilization. HB101(pMCG898) produced approximately 48% of the gluconic acid produced by *E. herbicola*.

The role of PQQ in many NADP-independent periplasmic dehydrogenases has been firmly established with gram-negative bacteria (1, 2, 5, 6). In both *Pseudomonas* species and *A. calcoaceticus*, membrane-bound GDH (EC 1.1.99.17) contains PQQ as a prosthetic group so that the functional holoenzyme is a quinoprotein (5, 6, 20).

It is known that *E. coli* K-12 synthesizes the apo-GDH enzyme and can use PQQ in medium to oxidize glucose to gluconic acid. Exogenous PQQ is effective because the GDH is located on the periplasmic side of the inner membrane. Goosen et al. have previously isolated a DNA fragment containing four genes in *A. calcoaceticus* essential for PQQ synthesis (9). The expression of these genes in *E. coli* K-12 leads to the synthesis of PQQ and glucose oxidation. On the basis of the observed production of gluconic acid and the strong sequence homology, it is reasonable to propose that the *E. herbicola* DNA fragment responsible for gluconic acid production and the Mps⁺ phenotype is functioning as a PQQ synthase gene in *E. coli* HB101. Goosen et al. have previously postulated that a precursor resembling the mature coenzyme is already present in *E. coli* so that only a small number of additional genes are required for PQQ synthesis (9).

Although we did not assay for PQQ directly, only recombinants containing an undisrupted 1.8-kb region of pMCG898 produced gluconic acid, which strongly implied the biosynthesis of PQQ and the assembly of the holo-GDH. With the exception of the DNA sequence shown in Fig. 4, no open reading frame longer than a couple hundred bases was identified in this 1.8-kb region. Assuming that the *Erwinia* gene identified in this study is similar in function to gene III of the four-gene PQQ synthase complex of *A. calcoaceticus* identified previously by Goosen et al. (9), the question of why, apparently, a single gene is sufficient in our system remains. One possibility is that some strains of *E. coli* contain cryptic PQQ synthase genes. There has been at least one report of the deblocking of PQQ synthase genes in *E. coli* (3). Should this be the case, it is reasonable to assume that different strains of *E. coli* may carry some but not all of these cryptic PQQ synthase genes. Goosen et al. (9) used *E. coli* K-12, whereas we have used strain HB101, an *E. coli* K-12-B hybrid (16). We note that preliminary Southern blots using the insert of pMCG898 as the probe did not show any homology to *E. coli* B or HB101 DNA (data not shown).

On the basis of these data, we propose that we have cloned and characterized a PQQ synthase gene from *E. herbicola* and that gluconic acid produced via the GDH-PQQ holoenzyme provides a molecular basis for the Mps⁺ phenotype shown by *E. herbicola* and recombinant *E. coli*. In many gram-negative bacteria, gluconic acid may undergo further extracellular oxidation to 2,5-diketogluconic acid (2). These pathways may provide the basis for the metabolic engineering of the Mps⁺ phenotype to accomplish efficacious biotransformation of rock phosphates.

ACKNOWLEDGMENTS

This work was supported by biological research grant NSC78-0418-B169-03 to S.T.L. from the National Science Council of the Republic of China and by research contract DEAC07-76ID01570 to J.H.W., R.R., and A.H.G. from the USDOE Office of Industrial Processes, Division of Advanced Industrial Concepts.

REFERENCES

- Ameyama, M., K. Matsushita, Y. Ohno, E. Shinagawa, and O. Adachi. 1981. D-Glucose dehydrogenase of *Gluconobacter suboxydans*: solubilization, purification and characterization. *Agric. Biol. Chem.* 45:851-861.
- Anderson, S., C. B. Marks, R. Lazarus, J. Miller, K. Stafford, J. Seymour, D. Light, W. Rastetter, and D. Estell. 1985. Production of 2-keto-L-gluconate, an intermediate in L-ascorbate synthesis, by a genetically modified *Erwinia herbicola*. *Science* 230:144-149.
- Biville, F., E. Turlin, and F. Gasser. 1991. Mutants of *Escherichia coli* producing pyrroloquinoline quinone. *J. Gen. Microbiol.* 137:1775-1783.
- Boyer, H. W., and D. Roulland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *Mol. Biol.* 41:459-472.
- Duine, J. A. 1991. Quinoproteins: enzymes containing the quinoid cofactor pyrroloquinoline quinone, topaquinone or tryptophan-tryptophan quinone. *Eur. J. Biochem.* 200:271-284.
- Duine, J. A., J. Frank, and J. K. Van Zeeland. 1979. Glucose dehydrogenase from *Acinetobacter calcoaceticus*, a 'quinoprotein.' *FEBS Lett.* 108:443-446.
- Goldstein, A. H. 1986. Bacterial mineral phosphate solubilization: historical perspectives and future prospects. *Am. J. Alt. Agric.* 1:57-65.
- Goldstein, A. H., and S. T. Liu. 1987. Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. *Bio/Technology* 5:72-74.
- Goosen, N., H. P. Horsman, R. G. Huinen, and P. van de Putte. 1989. *Acinetobacter calcoaceticus* genes involved in biosynthesis of the coenzyme pyrrolo-quinoline-quinone: nucleotide sequence and expression in *Escherichia coli* K-12. *J. Bacteriol.* 171:447-455.
- Guyer, M. S. 1978. The gamma-delta sequence of F is an insertion sequence. *J. Mol. Biol.* 126:347-365.
- Intelligenetics, Inc. 1991. Intelligenetics Suite, release 5.4. Intelligenetics, Inc., Mountain View, Calif.
- Kado, C. I., and S.-T. Liu. 1981. A rapid procedure for determination and isolation of large and small plasmids. *J. Bacteriol.* 145:1365-1373.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Lee, L.-Y., and S.-T. Liu. 1991. Characterization of the yellow-pigment genes of *Erwinia herbicola*. *Mol. Microbiol.* 5:217-224.
- Liu, L., W. Whalen, A. Das, and C. M. Berg. 1987. Rapid screening of cloned DNA using a transposon for bidirectional priming: sequence of the *Escherichia coli* K12 *avtA* gene. *Nucleic Acids Res.* 15:9461-9469.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Meagher, R. B., R. C. Tait, M. Betlach, and H. W. Boyer. 1977.

- Protein expression in *E. coli* minicells by recombinant plasmids. *Cell* **10**:521–536.
18. Perry, K. L., T. A. Simonitch, K. J. Harrison-Lavoie, and S.-T. Liu. 1986. Cloning and regulation of *Erwinia herbicola* pigment genes. *J. Bacteriol.* **168**:607–612.
 19. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 20. van Schie, B. J., J. P. van Dijken, and J. G. Kuenen. 1984. Non-coordinated synthesis of glucose dehydrogenase and its prosthetic group PQQ in *Acinetobacter* and *Pseudomonas* species. *FEMS Microbiol. Lett.* **24**:133.