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Sequencing and Expression of a Gene Encoding a Bile Acid Transporter from *Eubacterium* sp. Strain VPI 12708

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***Eubacterium* sp. strain VPI 12708 expresses inducible bile acid 7α-dehydroxylation activity via a multistep pathway. The genes encoding several of the inducible proteins involved in the pathway have been previously mapped to a bile acid-inducible (*bai*) operon in *Eubacterium* sp. strain VPI 12708. We now report the cloning, sequencing, and characterization of the *baiG* gene, which is part of the *bai* operon. The predicted amino acid sequence of the BaiG polypeptide shows significant homology to several membrane transport proteins, including sugar and antibiotic resistance transporters, which are members of the major facilitator superfamily. Hydrophilicity plots of BaiG show a high degree of similarity to class K and L TetA proteins from gram-positive bacteria, and, like these classes of TetA proteins, BaiG has 14 proposed transmembrane domains. The *baiG* gene was cloned into *Escherichia coli* and shown to confer an energy-dependent bile acid uptake activity. Primary bile acids were preferentially transported into *E. coli* cells expressing this gene, with at least sevenfold and fourfold increases in the uptake of cholic acid and chenodeoxycholic acid, respectively, over control reactions. Less transport activity was observed with cholylglycine, 7-oxocholic acid, and deoxycholic acid. The transport activity was inhibited by the proton ionophores carbonyl cyanide *m*-chlorophenylhydrazone, 2,4-dinitrophenol, and nigericin but not by the potassium ionophore valinomycin, suggesting that the transport is driven by the proton motive force across the cell membrane. In summary, we have cloned, sequenced, and expressed a bile acid-inducible bile acid transporter from *Eubacterium* sp. strain VPI 12708. To our knowledge, this is the first report of the cloning and expression of a gene encoding a procaryotic bile acid transporter.**

Eubacterium sp. strain VPI 12708 is an anaerobic intestinal bacterium which possesses bile acid 7α- and 7β-dehydroxylation activity (18, 40). The bile acid 7α-dehydroxylation activity is induced by unconjugated C_{24} bile acids which possess a 7α-OH group and is accompanied by the appearance of several new polypeptides on gels of soluble protein extracts from this bacterium (30, 39). The genes encoding several of these induced polypeptides have been previously mapped to a large bile acid-inducible (*bai*) operon in *Eubacterium* sp. strain VPI 12708 (10, 24). The *bai* operon contains at least nine open reading frames (Fig. 1) and is assumed to encode a majority of the proteins involved in the bile acid 7α-dehydroxylation pathway. The complete DNA sequences of *baiB*, *baiC*, *baiE*, *baiA2*, *baiF*, and *baiH* have been reported (10, 24, 41, 42).

In the 7α-dehydroxylation pathway (5), the primary bile acids cholic acid and chenodeoxycholic acid are 7α-dehydroxylated to deoxycholic acid and lithocholic acid, respectively. The pathway is initiated by conjugation of the primary bile acid to coenzyme A (22). The bile acid-coenzyme A conjugate is sequentially oxidized to a 3-oxo (23) and then a 3-oxo-Δ^4 bile acid intermediate. Following a 7α-dehydration step to a 3-oxo-$\Delta^{4,6}$ intermediate, in which the 7α-hydroxyl group is lost (7), there are three sequential reduction steps resulting in the final 7α-dehydroxylated bile acid. The physiological role of bile acid 7-dehydroxylation in the bacterial cell is unknown. However, it has been suggested that bile acid 7-dehydroxylation provides the bacterium with an ancillary electron acceptor (18).

The 7α-dehydroxylation reaction is quite rapid, in one study producing 17 nmol of deoxycholic acid/min/mg of protein when cholic acid-induced whole cells of *Eubacterium* sp. strain VPI 12708 were incubated in a reaction mixture containing 100 μM [24-^{14}C]cholic acid (40). However, neither induced nor uninduced cells show appreciable accumulation of cholic acid or its metabolites (39). This suggests the possibility of a constitutive bile acid exporter and raises the question whether *Eubacterium* sp. strain VPI 12708 has an inducible import mechanism for bile acids. In this paper, we report the cloning, expression, and characterization of an inducible bile acid transporter from *Eubacterium* sp. strain VPI 12708.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. *Escherichia coli* DH5α (Life Technologies, Gaithersburg, Md.) was used as the host strain for recombinant plasmids based on plasmid pSport1 (Life Technologies). The *E. coli* strains were grown on LB medium (1% tryptone [Difco Laboratories, Detroit, Mich.], 1% yeast extract, 0.5% NaCl [pH 7.4]) supplemented with 100 μg of ampicillin per ml as required.

Sequencing of the *baiG* gene. Due to difficulties encountered in obtaining clones in this region of the operon (24), the 5′ region of the *baiG* gene was sequenced from DNA fragments obtained by PCR. A 335-bp *Hae*III fragment and a 597-bp *Bcl*I fragment, both of which overlap the adjoining *baiF* gene by approximately 200 bp, were amplified by inverse PCR and sequenced as previously described (24). The 3′ end of the *baiG* gene was sequenced from a previously reported 1.8-kb *Eco*RI fragment, which also contained a large portion of the adjoining *baiH* gene (10). The middle portion of the *baiG* gene was amplified by PCR from *Eubacterium* sp. strain VPI 12708 chromosomal DNA with primers which created a 934-bp fragment that overlapped the previously mentioned *Bcl*I site on the 5′ end and the *Eco*RI site on the 3′ end. The amplified DNA fragment was then subjected to asymmetric PCR before being sequenced. All DNA fragments were sequenced completely from both strands.

Construction of a *baiG* expression vector. The *baiG* gene was amplified by PCR from *Eubacterium* sp. strain VPI 12708 chromosomal DNA with the synthetic oligonucleotide primers 5′-CTGGTACCGAGGAGTAAGAGTATGAG CAC and 5′-CCGGATCCTTATGCCTCTTTCTTCTGATAG, which add *Kpn*I and *Bam*HI sites, respectively. The resulting PCR product was digested with *Kpn*I and *Bam*HI and inserted into a predigested pSport1 vector. The 1,447-bp insert portion of the resulting plasmid (pSport1-50K) was sequenced to verify the fidelity of the PCR. This plasmid construct allows expression of *baiG* through the

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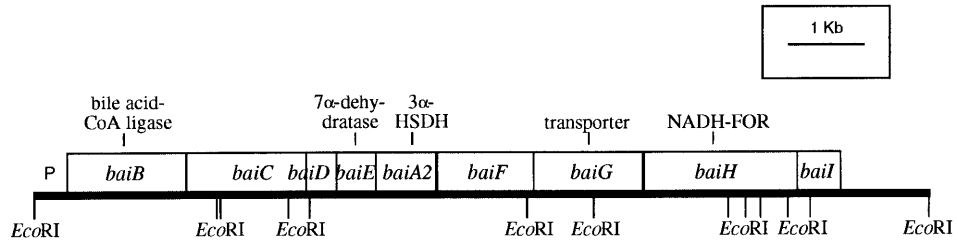

FIG. 1. The *bai* operon from *Eubacterium* sp. strain VPI 12708 (10, 24).

isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible T7 and *lac* promoters in the pSport1 vector.

Analysis of sequence data. Amino acid sequence analyses were performed with the Wisconsin Package (Genetics Computer Group, Madison, Wis.). The FASTA algorithm was used to find sequences homologous to the BaiG polypeptide. The PileUp and LineUp programs were used for multiple-sequence alignment and editing, respectively. The Jukes-Cantor distance and Kimura protein distance methods were used to create distances matrices based on the PileUp alignments, and the UPGMA and neighbor-joining algorithms were used to create phylogenetic trees from the Jukes-Cantor distance or Kimura protein distance files.

Bile acid nomenclature. The bile acid nomenclature used in this paper follows the recommendations of Hofmann et al. (17).

Radiolabeled bile acids. Radiolabeled [24-^{14}C]cholic acid, [24-^{14}C]chenodeoxycholic acid, and [24-^{14}C]cholylglycine were purchased from Dupont NEN (Boston, Mass.). Radiolabeled [24-^{14}C]deoxycholic acid and 7-[24-^{14}C]oxocholic acid were prepared by growth of *Eubacterium* sp. strain VPI 12708 in the presence of [24-^{14}C]cholic acid (40). Under the conditions used, the primary cholic acid metabolites are deoxycholic acid and 7-oxocholic acid. The resulting bile acids were extracted with ethyl acetate from the culture medium and separated with authentic deoxycholic acid and 7-oxocholic acid controls on silica 1B thin-layer chromatography plates (J. T. Baker, Inc., Phillipsburg, N.J.) with solvent system S4 (8). The plates were dried and exposed to X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.). Unlabeled controls were visualized with phosphotungstic acid (Sigma Chemical Co., St. Louis, Mo.). The ^{14}C-labeled bile acids were scraped from the plates and extracted with ethyl acetate, and the radioactivity was quantitated in a scintillation counter.

Transport assays. For bile acid transport studies, *E. coli* DH5α containing the pSport1-50K plasmid or a pSport1 control plasmid were grown with shaking at 37°C in LB medium containing 100 μg of ampicillin per ml to a Klett reading of 80. Cells containing the pSport1-50K plasmid were then induced with 0.4 mM IPTG and incubated for an additional 1.5 h. The cultures were adjusted to a Klett reading of 80, and 5-ml samples of cells were harvested by centrifugation at 3,000 × *g* for 10 min at room temperature. The cell pellets were resuspended in 5 ml of 50 mM Tris-HCl (pH 7.5) and centrifuged at 3,000 × *g* for 10 min at 4°C. The resulting cell pellets were resuspended in 500 μl of 50 mM potassium phosphate (pH 7.0) and preincubated at 37°C for 7 to 8 min. Reaction mixtures were prepared by adding 50 μl of the preincubated cells to 0.01 μCi of ^{14}C-labeled bile acid (final concentration, 4 μM). The reaction mixtures were scaled up as necessary. After the appropriate period of incubation at 37°C, 50-μl samples were collected, added to 1 ml of 100 mM LiCl–100 mM potassium phosphate (pH 7.0), and immediately filtered through Gelman GN-6, 25-mm, 0.45-μm-pore-size filters. The filters were washed twice with 5 ml of 100 mM LiCl–100 mM potassium phosphate (pH 7.0) and dried, and the radioactivity was quantitated in a scintillation counter. Background counts obtained from filtering the appropriate ^{14}C-labeled bile acid without cells were subtracted from all readings. Ionophores, when used, were added immediately before the 7- to 8-min preincubation period at the following concentrations: carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 10 μM; valinomycin, 1 μM; nigericin, 0.5 μM; and 2,4-dinitrophenol, 1 mM.

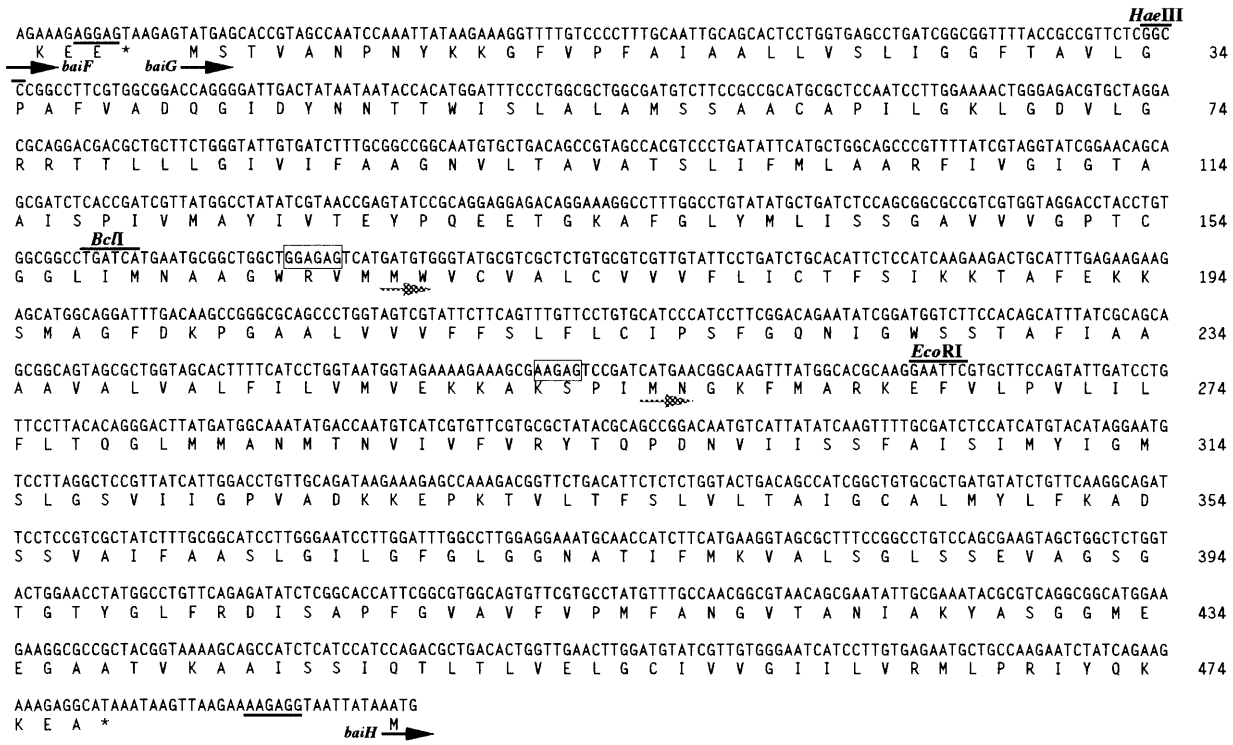

FIG. 2. Nucleotide and translated amino acid sequence of the *baiG* gene and surrounding region. Putative ribosome-binding sites are underlined. Possible internal ribosome-binding sites are boxed.

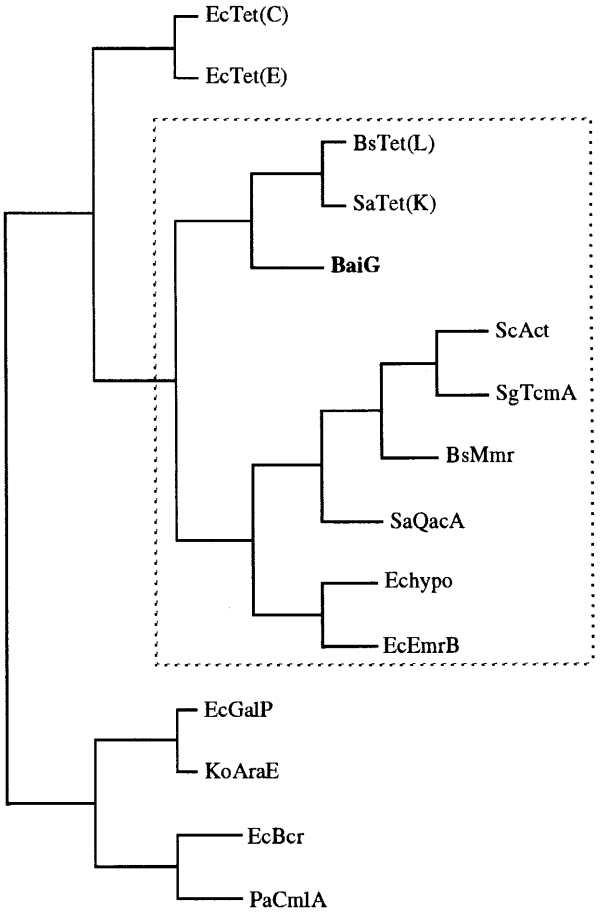

FIG. 3. Possible evolutionary tree for the BaiG protein and related proteins. The dotted box indicates the proteins most closely related to BaiG. Amino acid residues 50 to 164 of BaiG were used for FASTA database similarity searches. Homologous sequences were aligned with PileUp and manually edited with LineUp. The tree was constructed by using the UPGMA algorithm on a Kimura protein distance matrix. Sequences used: EcTet(C), *E. coli* TetA (class C) (31); EcTet(E), *E. coli* TetA (class E) (1); BsTet(L), *B. subtilis* TetA (class L) (29); SaTet(K), *Staphylococcus aureus* TetA (class K) (27); BaiG; ScAct, *Streptomyces coelicolor* actinorhodin resistance (9); SgTcmA, *Streptomyces glaucescens* tetracenomycin C resistance (11); BsMmr, *B. subtilis* methylenomycin A resistance (32); SaQacA, *S. aureus* antiseptic resistance (33); Echypo, *E. coli* hypothetical 51.5-kDa polypeptide (4); EcEmrB, *E. coli* multidrug resistance (21); EcGalP, *E. coli* galactose transporter (14); KoAraE, *Klebsiella oxytoca* arabinose transporter (36); EcBcr, *E. coli* bicyclomycin resistance (2); PaCmlA, *Pseudomonas aeruginosa* chloramphenicol resistance (3).

Nucleotide sequence accession number. The GenBank accession number of the sequence of the *baiG* gene and the rest of the *bai* operon is U57489 (replaces GenBank numbers M36292 and L11069).

RESULTS

Cloning and sequencing of *baiG*. The *baiG* gene from *Eubacterium* sp. strain VPI 12708 was sequenced from a combination of overlapping PCR fragments and clones as detailed in Materials and Methods. The gene contains an open reading frame capable of encoding a 477-amino-acid BaiG polypeptide with a calculated molecular weight of 49,964. The complete sequences of the *baiG* gene and BaiG polypeptide are presented in Fig. 2. A PCR was then used to amplify the entire *baiG* gene, and this fragment was cloned into a pSport1 expression vector, resulting in the pSport1-50K plasmid. Northern blots confirmed transcription of the *baiG* gene in IPTG-induced *E. coli* DH5α containing the pSport1-50K plasmid (data not presented). Several unsuccessful attempts were made to confirm the expression of BaiG with protein gels by using soluble extracts and membrane fractions from IPTG-induced cells of *E. coli* containing the pSport1-50K plasmid.

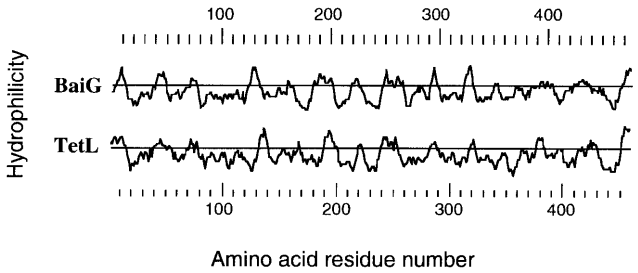

FIG. 4. Comparison of hydrophilicity plots for BaiG protein and TetA (class L) protein from *B. subtilis* (29).

Analysis of sequence data. Database searches revealed that the BaiG polypeptide shares sequence homology with a group of membrane proteins known as the major facilitator superfamily (25). These membrane proteins have been categorized as being composed of N-terminal (α) and C-terminal (β) domains (20). When an alignment of homologous sequences to amino acid residues 50 to 164 of BaiG (part of the α domain) is made, a possible evolutionary tree can be constructed (Fig. 3). An alignment in this region, in which the sequences that make up the tree have 25 to 35% amino acid sequence identity with BaiG, can be made with minimal manual editing of sequence gaps.

With the methods detailed in Fig. 3 or multiple-sequence alignments of entire polypeptides (data not shown), the sequences most closely related to BaiG are tetracycline resistance proteins from gram-positive bacteria, and several other prokaryotic drug resistance proteins (dotted box in Fig. 3). Figure 4 shows a comparison of hydropathy plots of the BaiG polypeptide and the TetA (class L) tetracycline resistance protein from *Bacillus subtilis*, and Fig. 5 presents a model of BaiG with 14 proposed transmembrane domains.

Development of a bile acid transport assay. Tetracycline transport assays used by Thanassi et al. (38) and McMurry et al. (26) were used as the starting point for development of a bile acid transport assay using [^{14}C]cholic acid in *E. coli*. The use of a potassium phosphate reaction buffer provided higher transport activity than did the use of sodium phosphate or Tris buffers. Incubation of the reaction mixtures at 37°C provided better transport activity than did incubation at 25, 32, or 42°C. The addition of 5 mM glucose, succinate, or citrate to the reaction mixtures did not enhance bile acid transport activity. The addition of 1 mM $CaCl_2$, $MgCl_2$, or $ZnCl_2$ had no apparent effect on bile acid transport activity. The accumulation of [^{14}C]cholic acid increased with decreasing pH in *E. coli* DH5α containing either the pSport1-50K plasmid or the pSport1 control plasmid. Assays performed at pH 7.0 provided good cholic acid transport activity in IPTG-induced cells containing the pSport1-50K plasmid and low background accumulation of cholic acid in control reactions with cells containing the pSport1 plasmid.

Bile acid transport studies. *E. coli* DH5α cells containing the pSport1-50K plasmid or a pSport1 control plasmid were tested for uptake of bile acids. Figure 6 shows that the uptake of ^{14}C-labeled cholic acid in IPTG-induced *E. coli* DH5α containing the pSport1-50K plasmid is markedly higher than in uninduced or control (pSport1 plasmid) cells. Table 1 presents

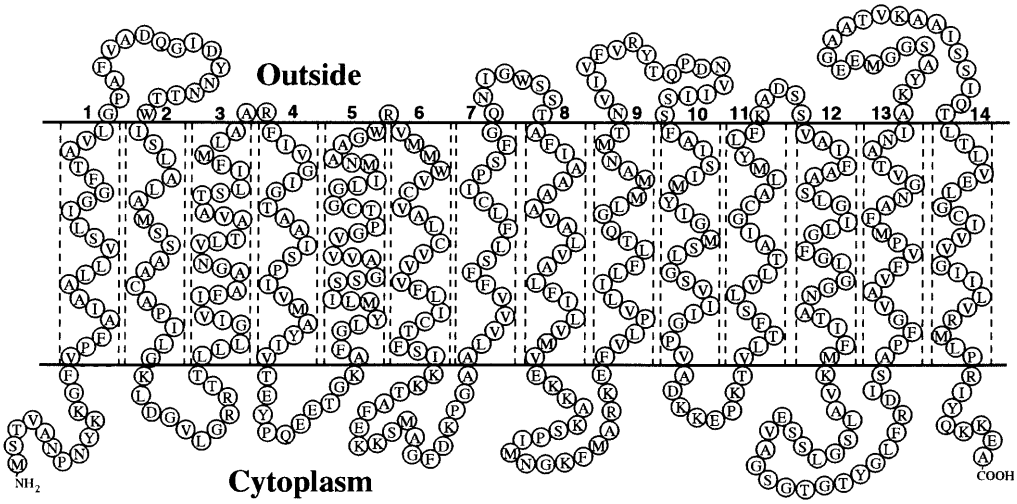

FIG. 5. Proposed model of transmembrane organization for BaiG polypeptide. The model was prepared from data obtained from the TMpred program through the ISREC TMpred server (http://ulrec3.unil.ch/software/TMPRED_form.html).

data on the uptake of several selected ^{14}C-labeled bile acids in IPTG-induced *E. coli* DH5α containing the pSport1-50K plasmid and in control cells containing only the pSport1 plasmid. The primary unconjugated bile acids cholic acid and chenodeoxycholic acid showed the greatest increase in uptake with the induced *E. coli* cells containing the pSport1-50K plasmid.

Effect of ionophores. The effect of ionophores on the uptake of bile acids by IPTG-induced *E. coli* DH5α containing the pSport1-50K plasmid was studied. The proton ionophores CCCP and 2,4-dinitrophenol reduced the uptake of cholic acid to 48.7 and 61.7%, respectively, of cholic acid uptake in control reactions with no additives. The proton/potassium antiporter nigericin reduced uptake activity to 63.7% of control activity, and addition of the potassium ionophore valinomycin resulted in 95.7% activity. Studying the effect of ionophores on cholic acid uptake in *E. coli* containing plasmid pSport1 without the *baiG* gene was difficult because the uptake was quite low. However, the addition of CCCP to these control reaction mixtures consistently produced higher levels of cholic acid uptake.

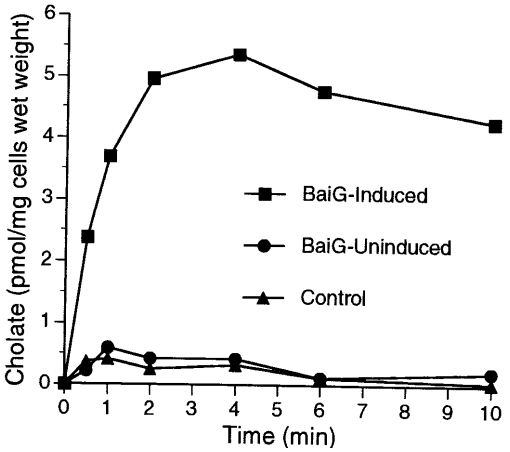

FIG. 6. Uptake of 4 μM [^{14}C]cholic acid into IPTG-induced and uninduced *E. coli* DH5α containing the pSport1-50K plasmid and into control cells containing the pSport1 plasmid.

DISCUSSION

We have shown that the *baiG* gene from *Eubacterium* sp. strain VPI 12708 encodes a 477-amino-acid polypeptide which has sequence homology to membrane transport proteins. The *baiG* gene is part of a large bile acid-inducible operon in *Eubacterium* sp. strain VPI 12708 which encodes polypeptides involved in the bile acid 7α-dehydroxylation pathway. The *baiG* gene confers an energy-dependent bile acid uptake activity on *E. coli* containing the cloned gene. Higher levels of uptake activity are observed with cholic acid and chenodeoxycholic acid than with deoxycholic acid, cholylglycine, or 7-oxocholic acid. Cholic acid and chenodeoxycholic acid are substrates for the bile acid-inducible 7α-dehydroxylation pathway and the constitutive 7α-hydroxysteroid dehydrogenase reaction in *Eubacterium* sp. strain VPI 12708. Deoxycholic acid is the product of the 7α-dehydroxylation pathway in *Eubacterium* sp. strain VPI 12708 when cholic acid is the substrate, and 7-oxocholic acid is the product of the constitutive 7α-hydroxysteroid dehydrogenase in this bacterium when cholic acid is the substrate. Cholylglycine is not a substrate for either the 7α-dehydroxylation reaction or 7α-hydroxysteroid dehydrogenase reaction, and *Eubacterium* sp. strain VPI 12708 has no bile salt hydrolase activity. Therefore, the results suggest that the function of the bile acid-inducible BaiG protein in *Eubacterium* sp. strain VPI 12708 is to import bile acids used in the 7α-dehydroxylation reaction.

The results also suggest that there must be a bile acid export activity in *Eubacterium* sp. strain VPI 12708 to remove the products of the 7α-dehydroxylation and 7α-hydroxysteroid dehydrogenase activities, as well as other bile acids that may accumulate in the cell. Thanassi and Nikaido (37) have shown that at least some strains of *E. coli* possess bile acid export activity. Data from the present study suggest that *E. coli* DH5α may possess some degree of bile acid export activity, as CCCP-treated cells displayed a small but consistent increase in accumulation of cholic acid when grown in the absence of the *baiG* gene. It is unclear how much effect, if any, this export activity may have had on the bile acid uptake results. The gradual reduction in the amount of cholic acid contained in the cells after the 4-min point in uptake reactions (Fig. 6) may also be related to bile acid export activity.

TABLE 1. Comparison of bile acid uptake by *E. coli* DH5α pSport1 (control) and *E. coli* DH5α pSport1-50K (BaiG)

Bile acid	Bile acid uptake (pmol)[a]			% Increase
	Control	BaiG	Increase	
Cholate	<1.0[b]	7.4	>6.4	>640
Chenodeoxycholate	6.5	28.7	22.2	342
Deoxycholate	3.9	8.9	5.0	128
Cholylglycine	2.1	3.2	1.1	52
7-Oxocholate	2.9	3.7	0.8	28

[a] Uptake of ^{14}C-labeled bile acids after a 4-min incubation of 1 mg (wet weight) of cells with 4 μM bile acid (n = 3). All standard deviations were less than 15%.

[b] Cholate uptake by control cells was too close to background for an accurate measurement.

It should be noted that bile acid uptake activity was not completely eliminated by addition of the proton ionophores. Parallel elimination of bile acid export activity may help explain these results. The cells expressing BaiG also had a sharp drop-off in growth following induction with IPTG; therefore, it is possible that part of the ionophore-insensitive increase in bile acid retention is due to the physiologically compromised state of the cells. A small across-the-board decrease in BaiG-induced bile acid uptake would also increase the apparent specificity of the bile acid transport activity (Table 1). A more detailed examination of the kinetics and specificity of bile acid transport by BaiG may require a different host and expression system.

The BaiG polypeptide has homology to the major facilitator superfamily of membrane transporters (25). Members of this superfamily of proteins which have the greatest homology to BaiG include bacterial sugar uptake proteins and antibiotic resistance proteins (Fig. 3). The most homologous of these are the class K and L tetracycline resistance proteins found in certain gram-positive bacteria (27, 29). These TetA (class K and L) proteins contain 14 proposed transmembrane domains, differing from the TetA proteins from gram-negative bacilli, which contain 12 proposed transmembrane domains (20).

It has been proposed (6, 20) that members of the major facilitator superfamily of transporters evolved from an ancestral polypeptide with six membrane-spanning regions (α domain). This ancestral polypeptide then combined through duplication or recombination with a β domain to form the present groups of polypeptides with 12 to 14 membrane-spanning regions. Examples of procaryotic proteins in this family with 12 proposed membrane-spanning regions include sugar transporters and various drug export proteins in addition to the tetracycline resistance factors from gram-negative bacilli (25, 28). A smaller group of proteins appear to have evolved with 14 proposed transmembrane regions. It is interesting that many of the proteins most closely related to BaiG (Fig. 3) are proposed to contain 14 membrane-spanning regions.

Studies have shown that while the individual α and β domains have no activity, domains from different classes of *E. coli* TetA proteins can be linked to form functional proteins (34) and separately expressed domains can interact to express reduced tetracycline resistance (35). These studies have suggested that these proteins exist as multimers in the membrane (15). There have also been reports on the possibility of internal translation initiation of functional β domains from genes encoding tetracycline resistance (16, 35). Interestingly, the *baiG* gene has at least two possible internal translation initiation sites which could theoretically produce polypeptides with 310 and 221 amino acids (Fig. 2). It is unclear if the *baiB* gene expresses these potential internal polypeptides, and the actual membrane organization of the active bile acid transporter has not been determined.

Like BaiG, most of the characterized bacterial transport proteins in the major facilitator superfamily rely on the proton motive force for transport activity. The tetracycline transporters are mostly tetracycline-divalent cation/proton antiporters. This transport activity consumes ΔpH but conserves $\Delta\Psi$, because the tetracycline molecule is negatively charged at physiological pH. The BaiG bile acid uptake system would be analogous in that, at least under the conditions tested (pH7), the symport of an ionized ($-COO^-$) bile acid and a proton would also consume ΔpH and preserve $\Delta\Psi$.

The BaiG polypeptide has no apparent homology to characterized mammalian bile acid transporters. A hamster ileal bile acid transporter (43) and human (12) and rat (13) liver bile acid transporters have been cloned and characterized. These transporters, which have substantial homology to each other, are Na^+/bile acid symporters, are proposed to have seven transmembrane domains, and have a common molecular weight of approximately 38,000. A rat liver Na^+-independent transporter has also been cloned and characterized (19). This transporter, which mediates transport activity for bile acids and other organic anions, has a molecular weight of 74,000 and is proposed to have 10 transmembrane domains.

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REFERENCES

1. **Allard, J. D., and K. P. Bertrand.** 1993. Sequence of a class E tetracycline resistance gene from *Escherichia coli* and comparison of related tetracycline efflux proteins. J. Bacteriol. **175:**4554–4560.
2. **Bentley, J., L. S. Hyatt, K. Ainley, J. H. Parish, R. B. Herbert, and G. R. White.** 1993. Cloning and sequence analysis of an *Escherichia coli* gene conferring bicyclomycin resistance. Gene **127:**117–120.
3. **Bissonnette, L., S. Champetier, J.-P. Buisson, and P. H. Roy.** 1991. Characterization of the nonenzymatic chloramphenicol resistance (*cmlA*) gene of the In*4* integron of Tn*1696*: similarity of the product to transmembrane transport proteins. J. Bacteriol. **173:**4493–4502.
4. **Burland, V., G. Plunkett III, D. L. Daniels, and F. R. Blattner.** 1993. DNA sequence and analysis of 136 kilobases of the *Escherichia coli* genome: organizational symmetry around the origin of replication. Genomics **16:**551–561.
5. **Coleman, J. P., W. B. White, M. Lijewski, and P. B. Hylemon.** 1988. Nucleotide sequence and regulation of a gene involved in bile acid 7-dehydroxylation by *Eubacterium* sp. strain VPI 12708. J. Bacteriol. **170:**2070–2077.
6. **Curiale, M. S., L. M. McMurry, and S. B. Levy.** 1984. Intracistronic complementation of the tetracycline resistance membrane protein of Tn*10*. J. Bacteriol. **157:**211–217.
7. **Dawson, J. A., D. H. Mallonee, I. Björkhem, and P. B. Hylemon.** Expression and characterization of a C_{24} bile acid 7α-dehydratase from *Eubacterium* sp. strain VPI 12708 in *Escherichia coli*. J. Lipid Res. **37:**1258–1267.
8. **Eneroth, P.** 1963. Thin-layer chromatography of bile acids. J. Lipid Res. **4:**11–16.
9. **Fernández-Moreno, M. A., J. L. Caballero, D. A. Hopwood, and F. Malpartida.** 1991. The *act* cluster contains regulatory and antibiotic export genes, direct targets for translational control by *bldA* tRNA gene of Streptomyces. Cell **66:**769–780.
10. **Franklund, C. V., S. F. Baron, and P. B. Hylemon.** 1993. Characterization of the *baiH* gene encoding a bile acid-inducible NADH:flavin oxidoreductase from *Eubacterium* sp. strain VPI 12708. J. Bacteriol. **175:**3002–3012.
11. **Guilfoile, P. G., and C. R. Hutchinson.** 1992. Sequence and transcriptional analysis of the *Streptomyces glaucescens tcmAR* tetracenomycin C resistance and repressor gene loci. J. Bacteriol. **174:**3651–3658.
12. **Hagenbuch, B., and P. J. Meier.** 1994. Molecular cloning, chromosomal localization, and functional characterization of a human liver Na^+/bile acid cotransporter. J. Clin. Invest. **93:**1326–1331.
13. **Hagenbuch, B., B. Stieger, M. Foguet, H. Lübbert, and P. J. Meier.** 1991. Functional expression cloning and characterization of the hepatocyte Na+/bile acid cotransport system. Proc. Natl. Acad. Sci. USA **88:**10629–10633.

14. **Henderson, P. J. F., S. A. Baldwin, M. T. C. Cairns, B. Charalambous, H. C. Dent, F. G. Gunn, W.-J. Liang, V. A. Lucas, G. E. M. Martin, T. P. McDonald, B. J. McKeown, J. A. R. Muiry, K. R. Petro, P. E. Roberts, K. P. Shatwell, G. Smith, and C. G. Tate.** 1992. Sugar-cation symport systems in bacteria. Int. Rev. Cytol. **137:**149–208.
15. **Hickman, R. K., and S. B. Levy.** 1988. Evidence that TET protein functions as a multimer in the inner membrane of *Escherichia coli*. J. Bacteriol. **170:** 1715–1720.
16. **Hillen, W., and K. Schollmeier.** 1983. Nucleotide sequence of the Tn*10* encoded tetracycline resistance gene. Nucleic Acids Res. **11:**525–539.
17. **Hofmann, A. F., J. Sjövall, G. Kurz, A. Radominska, C. D. Schteingart, G. S. Tint, Z. R. Vlahcevic, and K. D. R. Setchell.** 1992. A proposed nomenclature for bile acids. J. Lipid Res. **33:**599–604.
18. **Hylemon, P. B.** 1985. Metabolism of bile acids in intestinal microflora. New Compr. Biochem. **12:**331–343.
19. **Jacquemin, E., B. Hagenbuch, B. Stieger, and A. W. Wolkoff.** 1994. Expression cloning of a rat liver Na^+-independent organic anion transporter. Proc. Natl. Acad. Sci. USA **91:**133–137.
20. **Levy, S. B.** 1992. Active efflux mechanisms for antimicrobial resistance. Antimicrob. Agents Chemother. **36:**695–703.
21. **Lomovskaya, O., and K. Lewis.** 1992. *emr*, an *Escherichia coli* locus for multidrug resistance. Proc. Natl. Acad. Sci. USA **89:**8938–8942.
22. **Mallonee, D. H., J. L. Adams, and P. B. Hylemon.** 1992. The bile acid-inducible *baiB* gene from *Eubacterium* sp. strain VPI 12708 encodes a bile acid-coenzyme A ligase. J. Bacteriol. **174:**2065–2071.
23. **Mallonee, D. H., M. A. Lijewski, and P. B. Hylemon.** 1995. Expression in *Escherichia coli* and characterization of a bile acid-inducible 3α-hydroxysteroid dehydrogenase from *Eubacterium* sp. strain VPI 12708. Curr. Microbiol. **30:**259–263.
24. **Mallonee, D. H., W. B. White, and P. B. Hylemon.** 1990. Cloning and sequencing of a bile acid-inducible operon from *Eubacterium* sp. strain VPI 12708. J. Bacteriol. **172:**7011–7019.
25. **Marger, M. D., and M. H. Saier, Jr.** 1993. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem. Sci. **18:**13–20.
26. **McMurry, L., R. E. Petrucci, and S. B. Levy.** 1980. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77:**3974–3977.
27. **Mojumdar, M., and S. A. Khan.** 1988. Characterization of the tetracycline resistance gene of plasmid pT181 of *Staphylococcus aureus*. J. Bacteriol. **170:**5522–5528.
28. **Nikaido, H., and M. H. Saier, Jr.** 1992. Transport proteins in bacteria: common themes in their design. Science **258:**936–942.
29. **Ogasawara, N., S. Nakai, and H. Yoshikawa.** 1994. Systematic sequencing of the 180 kilobase region of the *Bacillus subtilis* chromosome containing the replication origin. DNA Res. **1:**1–14.
30. **Paone, D. A. M., and P. B. Hylemon.** 1984. HPLC purification and preparation of antibodies to cholic acid-inducible polypeptides from *Eubacterium* sp. VPI 12708. J. Lipid Res. **25:**1343–1349.
31. **Peden, K. W. C.** 1983. Revised sequence of the tetracycline resistance gene of pBR322. Gene **22:**277–280.
32. **Putzer, H., N. Gendron, and M. Grunberg-Manago.** Unpublished data.
33. **Rouch, D. A., D. S. Cram, D. DiBerardino, T. G. Littlejohn, and R. A. Skurray.** 1990. Efflux-mediated antiseptic resistance gene *qacA* from *Staphylococcus aureus*: common ancestry with tetracycline- and sugar-transport proteins. Mol. Microbiol. **4:**2051–2062.
34. **Rubin, R. A., and S. B. Levy.** 1990. Interdomain hybrid tet proteins confer tetracycline resistance only when they are derived from closely related members of the *tet* gene family. J. Bacteriol. **172:**2303–2312.
35. **Rubin, R. A., and S. B. Levy.** 1991. Tet protein domains interact productively to mediate tetracycline resistance when present on separate polypeptides. J. Bacteriol **173:**4503–4509.
36. **Shatwell, K. P., B. M. Charalambous, T. P. McDonald, and P. J. F. Henderson.** 1995. Cloning, sequencing, and expression of the *araE* gene of *Klebsiella oxytoca* 8017, which encodes arabinose-H^+ symport activity. J. Bacteriol. **177:**5379–5380.
37. **Thanassi, D. G., and H. Nikaido.** 1995. Active efflux of bile acids in *Escherichia coli*, abstr. H-249, p. 553. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
38. **Thanassi, D. G., G. S. B. Suh, and H. Nikaido.** 1995. Role of outer membrane barrier in efflux-mediated tetracycline resistance of *Escherichia coli*. J. Bacteriol. **177:**998–1007.
39. **White, B. A., A. F. Cacciapuoti, R. J. Fricke, T. R. Whitehead, E. H. Mosbach, and P. B. Hylemon.** 1981. Cofactor requirements for 7α-dehydroxylation of cholic and chenodeoxycholic acid in cell extracts of the intestinal anaerobic bacterium, *Eubacterium* species V.P.I. 12708. J. Lipid Res. **22:**891–898.
40. **White, B. A., R. L. Lipsky, R. J. Fricke, and P. B. Hylemon.** 1980. Bile acid induction specificity of 7α-dehydroxylase activity in an intestinal *Eubacterium* species. Steroids **35:**103–109.
41. **White, W. B., J. P. Coleman, and P. B. Hylemon.** 1988. Molecular cloning of a gene encoding a 45,000-dalton polypeptide associated with bile acid 7-dehydroxylation in *Eubacterium* sp. strain VPI 12708. J. Bacteriol. **170:**611–616.
42. **White, W. B., C. V. Franklund, J. P. Coleman, and P. B. Hylemon.** 1988. Evidence for a multigene family involved in bile acid 7-dehydroxylation in *Eubacterium* sp. strain VPI 12708. J. Bacteriol. **170:**4555–4561.
43. **Wong, M. H., P. Oelkers, A. L. Craddock, and P. A. Dawson.** 1994. Expression cloning and characterization of the hamster ileal sodium-dependent bile acid transporter. J. Biol. Chem. **269:**1340–1347.