Biochemical and Biophysical Research Communications **289,** 155–160 (2001)
doi:10.1006/bbrc.2001.5957, available online at http://www.idealibrary.com on IDEAL®

Essential Role of Residue H49 for Activity of *Escherichia coli* 1-Deoxy-D-xylulose 5-Phosphate Synthase, the Enzyme Catalyzing the First Step of the 2-*C*-Methyl-D-erythritol 4-Phosphate Pathway for Isoprenoid Synthesis

Jordi Querol, Manuel Rodríguez-Concepción, Albert Boronat, and Santiago Imperial[1]
Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Martí i Franquès 1-7, 08028 Barcelona, Spain

Received October 18, 2001

The first step of the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway for isoprenoid biosynthesis in plant plastids and most eubacteria is catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), a recently described transketolase-like enzyme. To identify key residues for DXS activity, we compared the amino acid sequence of *Escherichia coli* DXS with that of *E. coli* and yeast transketolase (TK). Alignment showed a previously undetected conserved region containing an invariant histidine residue that has been described to participate in proton transfer during TK catalysis. The possible role of the conserved residue in *E. coli* DXS (H49) was examined by site-directed mutagenesis. Replacement of this histidine residue with glutamine yielded a mutant DXS-H49Q enzyme that showed no detectable DXS activity. These findings are consistent with those obtained for yeast TK and demonstrate a key role of H49 for DXS activity.

Key Words: **1-deoxy-D-xylulose 5-phosphate synthase; isoprenoid biosynthesis; site-directed mutagenesis; transketolase.**

Isoprenoids are present in all living organisms and have important roles in membrane structure, redox chemistry, reproductive cycles, growth regulation, signal transduction, and defense mechanisms. Despite their functional and structural diversity, all isoprenoids derive from isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (1). Until recently it was accepted that all isoprenoids derived from mevalonic acid (MVA) synthesized by the condensation of three acetyl-CoA molecules. This well-known MVA pathway leads to the synthesis of IPP from MVA after phosphorylation and decarboxylation reactions (1). In many eubacteria (including *Escherichia coli*) and plant plastids, however, isoprenoids are synthesized through a completely different MVA-independent pathway, the so-called 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway (2–4). The first reaction of this pathway is the synthesis of 1-deoxy-D-xylulose 5-phosphate (DXP) by a condensation of (hydroxyethyl)thiamin derived from the decarboxylation of pyruvate with the C1 aldehyde group of D-glyceraldehyde 3-phosphate (GAP) (5, 6). This thiamin diphosphate (TPP) dependent reaction is catalyzed by the enzyme DXP synthase (DXS), a novel type of transketolase-like enzyme recently identified (7–9). In the next step, the enzyme DXP reductoisomerase (DXR) synthesizes MEP by intramolecular rearrangement and reduction of DXP (10–12). Subsequent reactions, catalyzed by the products of the *ygbB*, *ygbP* and *ychB* genes (13–16), led to the production of 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate, the proposed next intermediary of the pathway. The remaining enzymatic reactions eventually leading to the synthesis of the C5 isoprenoid building units remain to be fully characterized.

The MEP pathway is absent from yeast and animal cells, which utilize the MVA pathway for isoprenoid synthesis. In contrast, many pathogenic micro organisms only synthesize IPP and DMAPP through the MEP pathway. The enzymes of this pathway are therefore potential targets for the design of inhibitory drugs to be employed for the treatment of bacterial and parasitic infections, and also as herbicides. For instance, fosmidomycin and other derived specific inhibitors of DXR have already been used to inhibit isoprenoid synthesis in pathogenic bacteria and to block growth of the malaria parasite *Plasmodium falciparum* in infected mice (17).

Abbreviations used: DMAPP, dimethylallyl diphosphate; DX, 1-deoxy-D-xylulose; DXP, 1-deoxy-D-xylulose 5-phosphate; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; GAP, glyceraldehyde 3-phosphate; IPP, isopentenyl diphosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate: TK, transketolase; TPP, thiamine diphosphate.

[1] To whom correspondence and reprint requests should be addressed. Fax: (+34) 93 4021219. E-mail: imperial@bq.ub.es.

0006-291X/01 $35.00

In this context, DXS is a good candidate for the development of this new type of drugs for several reasons. First, the product of DXS activity, DXP, is a biosynthetic intermediate not only for isoprenoid synthesis but also for thiamine and pyridoxol synthesis (18, 19). In addition, the reaction catalyzed by DXS has been proposed as a key regulatory step for the biosynthesis of isoprenoid compounds in both bacteria and plants (20–22). Little is known, however, about the biochemistry of this enzyme. In the absence of structural and mechanistic information about DXS, the identification of essential amino acid residues for activity becomes critical. In the present work, we have carried out a comparison of the amino acid sequence of *E. coli* DXS with that of transketolase (TK, an extensively characterized enzyme catalyzing a similar TPP-dependent reaction) to search for residues with a potentially critical role for enzyme activity. As a result, we identified residue H49 as essential for DXS activity, and propose that, like H30 in yeast TK, this residue may be implicated in proton transfer during catalysis.

MATERIALS AND METHODS

Sequence analysis. Alignment of the amino acid sequences from *Escherichia coli* DXS (GenBank AF035440) and TK1 (Accession No. X68025) and *Saccharomyces cerevisiae* TK1 (X73224) was initially carried out with ClustalW (http://www2.ebi.ac.uk/clustalw/) using the default parameters. The output alignment was further refined by eye and edited using GeneDoc (http://www.psc.edu/biomed/genedoc/). Gaps were introduced into the sequences where necessary to improve the overall alignment, specially to allow identification of the conserved H49 residue and flanking region at the N-terminal domain of *E. coli* DXS.

Site-directed mutagenesis. Point mutation H49Q was introduced into the gene encoding *E. coli* DXS by site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Stratagene), *Pfu* DNA polymerase (Gibco-BRL) and primers DXSmut1 (5′-C C A G C G G G C A C T T C G C C T C C-3′) and DXSmut2 (5′-G G A G G C G A A G T G C C C G C T G G-3′). After digestion with *Nde*I and *Eco*RI, the DNA fragment encoding the full-length mutant protein DXS-H49Q was ligated into the same sites of the expression vector pTACTAC (23). The resulting plasmid was designated pTAC-DXS-H49Q. The mutation was confirmed by DNA sequencing with the ABI-PRISM system (PE Biosystems).

E. coli complementation assay. Cells from *E. coli* strain MC4100 *dxs::CAT*, carrying a disruption of the *dxs* gene with the *CAT* gene conferring resistance to chloramphenicol (24), were transformed with plasmids pTAC-ORF2 (8) (here designated pTAC-DXS), pTAC-DXS-H49Q, and pTACTAC using standard methods. Positive transformants were recovered on 2× TY plates supplemented with 0, 0.4, or 1 mM isopropyl β-D-thiogalactoside (IPTG), 100 μg/ml ampicillin, 34 μg/ml chloramphenicol, and 0.5 mM 1-deoxy-D-xylulose (DX) prepared as described (8). To determine growth rate, isolated colonies were used to inoculate 10 ml of liquid medium without DX. Aliquots of 1 ml were taken at the indicated time intervals for OD_{600} measurements.

Expression and activity of cloned DXS proteins. MC4100 *dxs::CAT* cells harboring plasmids pTAC-DXS, pTAC-DXS-H49Q, and pTACTAC were grown at 37°C in liquid 2× TY medium supplemented with 100 μg/ml ampicillin, 34 μg/ml chloramphenicol, and 0.5 mM DX to an OD_{600} of 0.6. Expression of the cloned proteins was induced by adding 0.4 mM IPTG to the medium. After 2 h, cells were harvested by centrifugation and resuspended in 40 mM Tris–HCl, pH 8.0, 2.5 mM $MgCl_2$, 1 mM TPP, 5 mM 2-mercaptoethanol, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml chymostatin, 1 mM Pefabloc (Roche) and 1 mg/ml lysozyme. Following incubation at 37°C for 20 min, cells were disrupted by sonication. Soluble fractions were obtained after centrifugation at 13,000*g* for 20 min at 4°C. Protein amount in the supernatant was quantified with the Bio-Rad protein assay. DXS activity was determined as described (8) with 10 μg of soluble protein sample in a final volume of 50 μl.

Immunoblot analysis. Aliquots of 0.5 μg of soluble protein were electrophoresed in 10% SDS–PAGE and electrotransferred to nitrocellulose membranes (Amersham) using the MiniProtean system (Bio-Rad). Blots were blocked in PBS-T (PBS buffer supplemented with 2% [w/v] powdered nonfat dry milk and 0.05% [v/v] Tween 20) for 18 h at 4°C. Incubation with an anti-DXS polyclonal antibody (25) diluted 1:500 in PBS-T was carried out for 1 h at room temperature. Blots were then washed 3 times for 10 min in PBS-T and incubated for 1 h at room temperature with an HRP-conjugated secondary antibody (Roche) diluted 1:10,000 in PBS-T. After 3 washes, immunodetection of cross-reacting bands was performed with the ECL system (Amersham).

RESULTS AND DISCUSSION

To identify key residues for DXS activity, we carried out a comparative study of the amino acid sequence of *E. coli* DXS with that of transketolase (TK), a similar TPP-dependent enzyme. TK participates, together with transaldolase, in the sugar rearrangement system acting in the nonoxidative part of the pentose-phosphate pathway and, in plants, participating in the regeneration of ribulose-1,5-bisphosphate from phosphoglycerate in the Calvin cycle (26). TK catalyzes two key reversible reactions that involve the condensation of a dihydroxyethyl group from the ketose donor with the C_1 aldehyde group of the aldose acceptor substrate. Crystallography and mutagenesis of yeast TK have provided a strong framework to which studies of other TPP-binding enzymes can be related, including the identification of specific residues involved in TPP-dependent enzymatic catalysis (26, 27). Our rationale was that conserved residues in the *E. coli* DXS sequence might have a similar role.

The alignment of *E. coli* DXS with TK sequences from *E. coli* and yeast shows conserved residues that are distributed along the protein sequences (Fig. 1). There are three regions, however, where sequence similarities are concentrated. These conserved domains have also been reported to be present in all the TK sequences analyzed so far, giving rise to the description of consensus sequences (28,29). One of such regions is the TPP-binding domain, present in all TPP-dependent enzymes. The proposed consensus **G-D-G-(X)$_4$-G-(X)$_3$-E-(X)$_4$-A-(X)$_4$-**L-(X)$_7$-D-**X-N** (28) is found almost invariant in *E. coli* DXS (Fig. 1). The two other conserved regions have been considered to be signature domains for the proteins within the TK family (28, 29), and their presence in the DXS sequence is consistent with the TK-like nature of the reaction catalyzed by this en-

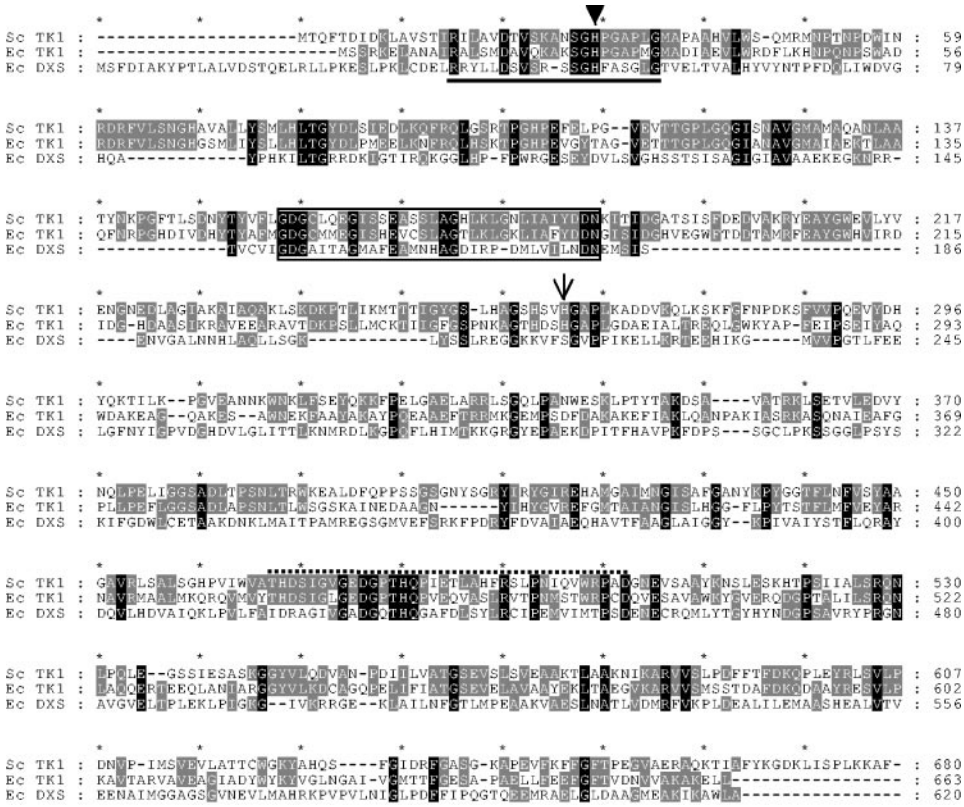

FIG. 1. Multiple alignment of TK and DXS proteins. Protein sequences from *Escherichia coli* DXS (GenBank AF035440), TK1 (Accession No. X68025), and *Saccharomyces cerevisiae* TK1 (X73224) were aligned using Clustal W. Alignment was later refined by eye, especially to allow identification of the conserved H49 residue (marked with an arrowhead) and its flanking region at the N-terminal domain of *E. coli* DXS (underlined). The TPP-binding domain is boxed, and the TK motif is marked with a dashed line. Amino acid residues conserved in all sequences are indicated in white inside black boxes. Residues conserved in only two sequences are marked in white inside gray boxes. The H263 residue in yeast TK1 sequence is indicated with an arrow.

zyme. One of these regions in *E. coli* DXS partially fits the so-called "TK motif" consensus [T,S]-H-[D,C]-[S,G]-X-[G,S,N,Q,A]-X-**G**-[E,G]-[**D**,N]-**G**-P-[**T**,S]-[**H**,Q]-X-[P,A]-X-[E,D]-$(X)_5$-**R**-$(X)_8$-[R,Y]-**P-X-D** (26,29). An additional region in the N-terminal domain of *E. coli* DXS showing a good match with the proposed TK consensus **R**-$(X)_4$-[**D**,E,Q]-$(X)_{5,6}$-[G,**S**,T]-**G-H**-[P,L,I,V,M]-[G,S,T]-$(X)_3$-[**G**,S,T] (28) was not detected in a previous alignment of DXS and TK sequences (30). This region contains a histidine residue perfectly conserved in all the members of the TK family as well as in all the known DXS enzymes analyzed so far (8). The role of this residue (H30) has been best characterized in yeast TK1, in which it has been reported to be involved in catalysis (26, 27, 31). H30 is localized in the active site of TK and appears to have a role in proton transfer during catalysis (27, 31). Besides H30, the histidine residue at position 263 (H263) in yeast TK is also localized in the active site of the enzyme and it was considered a second candidate for an acid/base catalyst in the reaction (26). It has been suggested that both histidine residues could act in concert at the active site of yeast TK during proton abstraction at the C3 atom of the donor substrate (31). Consistently, mutants at both positions are severely impaired in catalytic activity but unperturbed in TPP-binding capability (31). The histidine residue corresponding to yeast TK H30 is localized at position 49 (H49) in *E. coli* DXS (Fig. 1). Unlike H30, however, H263 appears not to be conserved in DXS (Fig. 1). The absence of an obvious equivalent to H263 in the DXS sequence is intriguing and suggests that H49 might be the only histidine residue strictly required for catalysis. Alternatively, the TK-like reaction catalyzed by DXS may involve different amino acid residues.

To study the role of the H49 residue for DXS activity, we constructed a mutant enzyme in which this conserved histidine was replaced by glutamine (termed DXS-H49Q mutant). The gene encoding *E. coli* DXS was used as a template for site-directed mutagenesis and the mutated version was cloned into the IPTG-inducible pTACTAC expression vector (23) to create plasmid pTAC-DXS-H49Q. The effect of the point mutation on DXS activity was tested *in vivo* using the DXS-deficient *E. coli* strain MC4100 *dxs::CAT* (24). This strain is unable to grow in the absence of an exogenously supplied IPP precursor (such as DX or

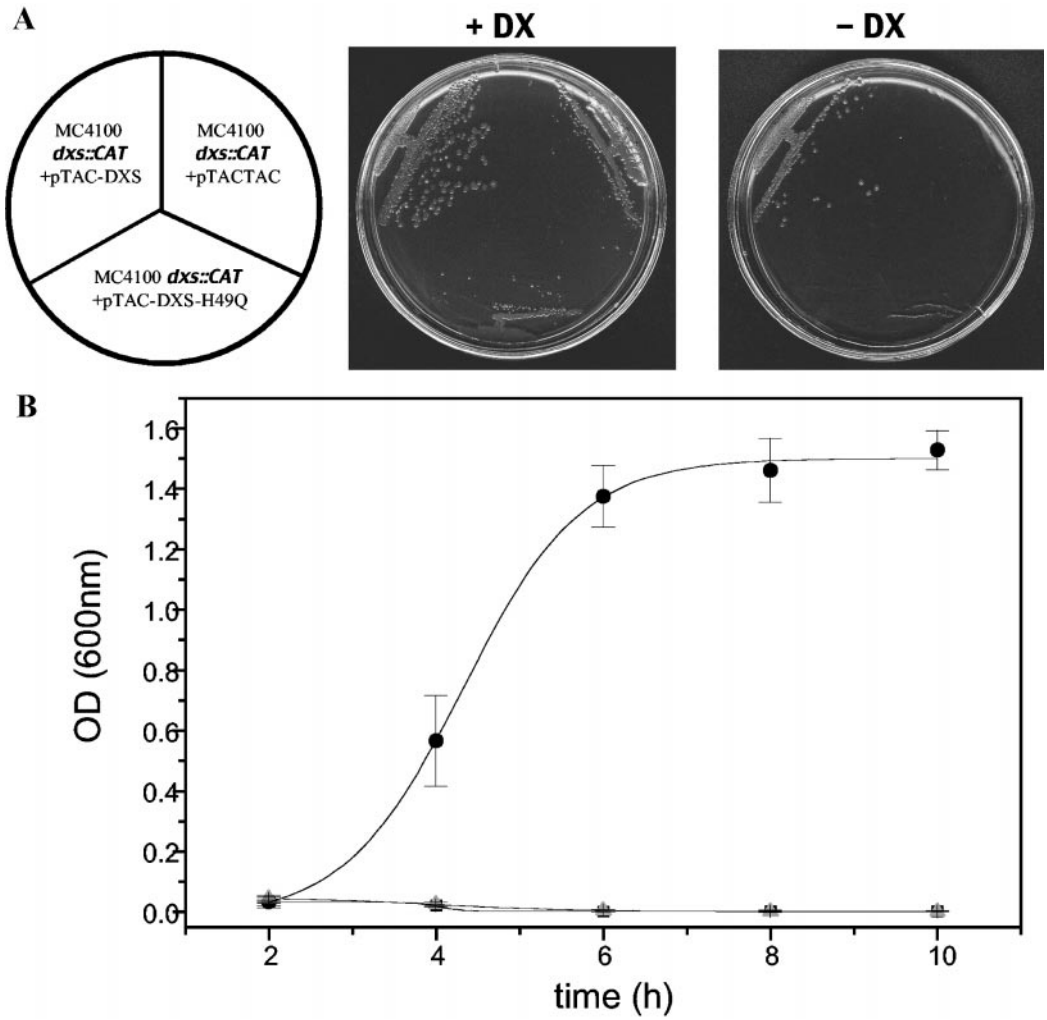

FIG. 2. Complementation of DXS-deficient *E. coli* cells. *E. coli* MC4100 *dxs::CAT* cells were transformed with pTACTAC, pTAC-DXS-H49Q, or pTAC-DXS and grown on 2× TY plates with or without 0.5 mM DX (A). Isolated colonies from DX-containing plates were used afterward to inoculate liquid medium without DX (B). Aliquots of culture were taken at the indicated times and growth was monitored by measuring absorbance at 600 nm. (■) pTACTAC; (▲) pTAC-DXS-H49Q; (●) pTAC-DXS.

methylerythritol) because the generated insertional disruption of the chromosomal *dxs* gene with the *CAT* selectable marker causes a complete block of isoprenoid synthesis (24). MC4100 *dxs::CAT* cells were transformed with plasmids pTACTAC, pTAC-DXS, pTAC-DXS-H49Q and plated on media with and without IPTG. Although similar results were obtained in all the cases, the presence of IPTG appeared to have a concentration-dependent inhibitory effect on bacterial growth (data not shown). Transformation of MC4100 *dxs::CAT* cells with plasmid pTAC-DXS-H49Q did not rescue DX auxotrophy (Fig. 2A). By contrast, the same strain was able to grow in the absence of DX when transformed with pTAC-DXS, a similar construct encoding the wild type *E. coli* DXS enzyme (8, 24) (Fig. 2A). Similar results were obtained when transformed MC4100 *dxs::CAT* cells were grown in liquid medium without DX. Whereas DXS-deficient cells carrying the pTAC-DXS plasmid grew normally in this medium, no growth was observed when the pTACTAC or pTAC-DXS-H49Q constructs were used (Fig. 2B), suggesting the possibility that the H49Q mutation might result in the loss of DXS enzyme activity. The results, however, could also be explained if DXS-H49Q was not expressed. Alternatively, the H49Q mutation may cause decreased protein stability compared to wild type DXS, altered folding, or accumulation of the mutant protein in inclusion bodies.

To find out whether the inability of the pTAC-DXS-H49Q construct to rescue DX auxotrophy of the DXS-deficient strain was due to decreased level or altered subcellular localization of the encoded DXS-H49Q protein compared to the wild type enzyme expressed from pTAC-DXS, we studied the distribution of both mutant and wild type enzymes in protein extracts by immunodetection with an anti-DXS antibody (25). MC4100

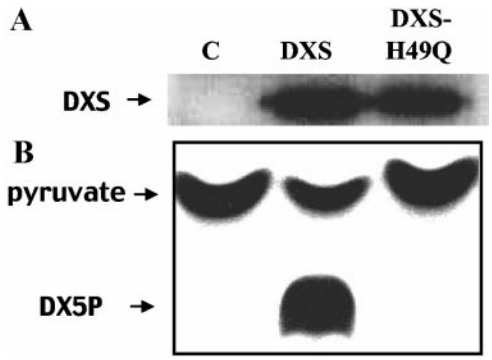

FIG. 3. Analysis of cloned DXS expression and activity. (A) *E. coli* MC4100 *dxs::CAT* strains harboring plasmids pTACTAC (C), pTAC-DXS-H49Q (DXS-H49Q), and pTAC-DXS (DXS) were grown on plates containing DX. Expression of the cloned DNAs was induced after transferring single colonies of the three strains to liquid media supplemented with 0.4 mM IPTG. Expressed proteins were detected by immunoblot analysis of 0.5 μg of soluble protein fractions with a specific anti-DXS antibody. (B) DXS activity determination. Cell-free extracts (10 μg of protein) were incubated for 1 h under the conditions described under Materials and Methods. Reaction mixture aliquots (5 μl) were analyzed by TLC. The position of the ^{14}C-labeled pyruvate substrate and DXP is indicated.

dxs::CAT cells transformed with either pTACTAC, pTAC-DXS-H49Q, or pTAC-DXS were grown in liquid medium supplemented with DX. After induction of cloned protein expression with 0.4 mM IPTG, a soluble protein extract was obtained from each strain and used for immunoblot analysis. Figure 3A shows that no protein was recognized by the anti-DXS antiserum in DXS-deficient cells transformed with the pTACTAC plasmid, as expected. By contrast, high levels of soluble DXS and DXS-H49Q were found in *E. coli* cells transformed with the corresponding vectors. No significant differences were found in the levels of mutant and wild type enzymes (Fig. 3A). This result shows that the H49Q mutation does not cause lower DXS protein levels or a major change in subcellular distribution. Together, the data strongly suggested that H49 may be crucial for the catalytic activity of *E. coli* DXS. To confirm whether the H49Q mutation indeed resulted in a severe reduction in enzyme activity, cell-free protein extracts obtained from induced MC4100 *dxs::CAT* cells harboring plasmids pTACTAC, pTAC-DXS-H49Q, or pTAC-DXS were used for DXS activity assays. Extracts were incubated with ^{14}C-labeled pyruvate and cold GAP as substrates. Afterwards, the reaction products were separated by TLC and analyzed by autoradiography (Fig. 3B). DXS activity was clearly found in DXS-deficient cells expressing the cloned wild type DXS. However, no detectable activity was present in MC4100 *dxs::CAT* cells expressing the DXS-H49Q mutant form or in the strain transformed with the control pTACTAC vector (Fig 3B).

Together, we have presented *in vivo* and *in vitro* evidence indicating that mutation of residue H49 in *E. coli* DXS results in a mutant enzyme with undetectable activity. Our results are consistent with those reported for H30 in yeast TK (31) and support that H30 in yeast TK and H49 in *E. coli* DXS are conserved equivalent residues which may play similar roles. This would involve that the observed loss of enzyme activity in DXS-H49Q most likely results from disturbed proton transfer during catalysis (26, 27, 31). The absence of an obvious conserved equivalent of yeast TK H263 residue in *E. coli* DXS, however, may argue against a requirement of two histidine residues acting in concert during catalysis, at least in DXS. Alternatively, histidine residues at positions 195 or 232 in *E. coli* DXS sequence (Fig. 1) might be the functional counterpart of H49. Further studies are needed therefore to establish whether the residues involved in catalysis are conserved in DXS. In the absence of a structural model obtained from X-ray crystallography, site-directed mutagenesis remains a powerful tool to identify essential residues for DXS enzyme activity and get a deeper insight into the biochemical mechanisms of this novel family of TK-like proteins.

ACKNOWLEDGMENTS

This work was supported in part by grants from Ferrer Internacional and the Generalitat de Catalunya (1999 SGR-00032). We thank Dr. Jaime F. Martínez-García for comments on the manuscript, Dr. Luisa M. Lois for material and advice, Dr. Josep L. Gelpí for helpful discussions, and Dr. Patricia León for the gift of the anti-DXS serum.

REFERENCES

1. Bartley, G. E., and Scolnik, P. A. (1995) Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. *Plant Cell* **7,** 1027–1038.
2. Lichtenthaler, H. K., Rohmer, M., and Schwender, J. (1997) Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. *Physiol. Plant.* **101,** 643–652.
3. Lichtenthaler, H. K. (1999) The 1-deoxy-D-xylulose 5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50,** 47–65.
4. Rohmer, M. (1999) The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Nat. Prod. Rep.* **16,** 565–574.
5. Arigoni, D., Sagner, S., Latzel, C., Eisenreich, W., Bacher, A., and ZenK, M. H. (1997) Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. *Proc. Natl. Acad. Sci. USA* **94,** 1600–1605.
6. Rohmer, M., Seemann, M., Horbach, S., Bringer-Meyer, S., and Sham, H. (1996) Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis. *J. Am. Chem. Soc.* **118,** 2564–2566.
7. Sprenger, G. A., Schörken, U., Wiegert, T., Grolle, S., De Graaf, A. A., Taylor, S. V., Begley, T. P., Bringer-Meyer, S., and Sahm, H. (1997) Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-

xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. *Proc. Natl. Acad. Sci. USA* **94,** 12857–12862.

8. Lois, L. M., Campos, N., Rosa Putra, S., Danielsen, K., Rohmer, M., and Boronat, A. (1998) Cloning and characterization of a gene from *Escherichia coli* encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxyxylulose 5-phosphate, a common precursor for isoprenoid, thiamin, and pyridoxol biosynthesis. *Proc. Natl. Acad. Sci. USA* **95,** 2105–2110.
9. Lange, B. M., and Croteau, R. (1999) Isoprenoid biosynthesis via a mevalonate-independent pathway in plants: Cloning and heterologous expression of 1-deoxy-D-xylulose 5-phosphate reductoisomerase from peppermint. *Arch. Biochem. Biophys.* **365,** 170–174.
10. Lange, B. M., Wildung, M. R., McCaskill, D., and Croteau, R. (1998) A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway. *Proc. Natl. Acad. Sci. USA* **95,** 2100–2104.
11. Schwender, J., Müller, C., Zeidler, J., and Lichtenthaler, H. K. (1999) Cloning and heterologous expression of a cDNA encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase of *Arabidopsis thaliana*. *FEBS Lett.* **455,** 140–144.
12. Takahashi, S., Kuzuyama, T., Watanabe, H., and Seto, H. (1998) A 1-deoxy-D-xylulose 5-phosphate reductoisomerase catalyzing the formation of 2-*C*-methyl-D-erythritol 4-phosphate in a an alternative nonmevalonate pathway for terpenoid biosynthesis. *Proc. Natl. Acad. Sci. USA* **95,** 9879–9884.
13. Rohdich, F., Wungsintaweekul, J., Fellermeier, M., Sagner, S., Herz, S., Kis, K., Eisenreich, W., Bacher, A., and Zenk, M. H. (1999) Cytidine 5′-triphosphate-dependent biosynthesis of isoprenoids: YgbP protein of *Escherichia coli* catalyzes the formation of 4-diphosphocytidil-2-*C*-methylerythritol. *Proc. Natl. Acad. Sci. USA* **96,** 11758–11763.
14. Herz, S., Wungsintaweekul, J., Schuhr, C. A., Hecht, S., Lüttgen, H., Sagner, S., Fellermeier, M., Eisenreich, W., Zenk, M. H., Bacher, A., and Rohdich, F. (2000). Biosynthesis of terpenoids: YgbB protein converts 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate to 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate. *Proc. Natl. Acad. Sci. USA* **97,** 2486–2490.
15. Kuzuyama, T. A., Takagi, M., Kaneda, K., Dairi, T., and Seto. H. (2000) Studies on the nonmevalonate pathway: Formation of 2-*C*-methyl-D-erythritol 2,5-cyclodiphosphate from 2-phospho-4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol. *Tetrahedron Lett.* **41,** 703–706.
16. Lüttgen, H., Rohdich, F., Herz, S., Wungsintaweekul, J., Hecht, S., Schuhr, C. A., Fellermeier, M., Sagner, S., Zenk, M. H., Bacher, A., and Eisenreich, W. (2000) Biosynthesis of terpenoids: YchB protein of *Escherichia coli* phosphorylates the 2-hydroxy group of 4-diphosphocytidyl-2-*C*-methyl-D-erythritol. *Proc. Natl. Acad. Sci. USA* **97,** 1062–1067.
17. Lichtenthaler, H. K., Zeidler, J., Schwender, J., and Müller, C. (2000) The non-mevalonate isoprenoid biosynthesis of plants as a test system for new herbicides and drugs against pathogenic bacteria and the malaria parasite. *Z. Naturforsch.* **55c,** 305–313.
18. Julliard, J. H., and Douce, R. (1991) Biosynthesis of the thiazole moiety of thiamin (vitamin B_1) in higher plant chloroplasts. *Proc. Natl. Acad. Sci. USA* **88,** 2042–2045.
19. Julliard, J. H. (1992) Biosynthesis of the pyridoxal ring (vitamin B_6) in higher plant chloroplasts and its relationship with the biosynthesis of the thiazole ring (vitamin B_1). *CR Acad. Sci. Ser. III* **314,** 285–290.
20. Harker, M., and Bramley, P. M. (1999) Expression of procaryotic 1-deoxy-D-xylulose 5-phosphatases in *Escherichia coli* increases carotenoid and ubiquinone biosynthesis. *FEBS Lett.* **448.** 115–119.
21. Lois, L. M., Rodríguez-Concepción, M., Gallego, F., Campos, N., and Boronat A. (2000) Carotenoid biosynthesis during tomato fruit development: Regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *Plant J.* **22,** 503–513.
22. Miller, B., Heuser, T., and Zimmer, W. (2000) Functional involvement of a deoxy-D-xylulose 5-phosphate reductoisomerase gene harboring locus of *Synechococcus leopoliensis* in isoprenoid biosynthesis. *FEBS Lett.* **481,** 221–226.
23. Browner, M. F., Rasor, P., Tugendreich, S., and Fletterick, R. J. (1991) Temperature-sensitive production of rabbit muscle glycogen phosphorylase in *Escherichia coli*. *Protein Eng.* **4,** 351–357.
24. Charon, L., Hoeffer, J. F., Pale-Grosdemange, C., Lois, L. M., Campos, N., Boronat, A., and Rohmer, M. (2000) Deuterium labeled isotopomers of 2-*C*-methyl-D-erythritol as tools for the elucidation of the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway for isoprenoid synthesis. *Biochem. J.* **346,** 737–742.
25. Estévez, J. M., Cantero, A., Romero, C., Kawaide, H., Jiménez, L. F., Kuzuyama, T., Seto, H., Kamiya, Y., and León, P. (2000) Analysis of the expression of *CLA1*, a gene that encodes the 1-deoxyxylulose 5-phosphate synthase of the 2-*C*-methyl-D-erythritol-4-phosphate pathway in *Arabidopsis. Plant Physiol.* **124,** 95–103.
26. Schneider, G., and Linvsqvist, Y. 1998. Crystallography and mutagenesis of transketolase: Mechanistic implications for enzymatic thiamin catalysis. *Biochim. Biophys. Acta* **1385,** 387–398.
27. Linvsqvist, Y., Schneider, G., Ermler, U., and Sundström, M. (1992) Three-dimensional structure of transketolase, a thiamin diphosphate dependent enzyme, at 2.5 Å resolution. *EMBO J.* **11,** 2373–2379.
28. Reizer, J., Reizer, A., Bairoch, A., and Saier, M. H. (1993) A diverse transketolase family that includes the RecP protein of *Streptococcus pneumoniae*, a protein implicated in genetic recombination. *Res. Microbiol.* **144,** 341–347.
29. Schenk, G., Layfield, R., Candy, J. M., Duggleby, R. G., and Nixon, P. F. (1997) Molecular evolutionary analysis of the thiamine-diphosphate-dependent enzyme, transketolase. *J. Mol. Evol.* **44,** 552–572.
30. Mandel, M. A., Feldmann, K. A., Herrera-Estrella, L., Rocha-Sosa, M., and León, P. (1996) *CLA1*, a novel gene required for chloroplast development, is highly conserved in evolution. *Plant J.* **9,** 649–658.
31. Wikner, C., Nilsson, U., Meshalkina, L., Udekwu, C., Lindqvist, Y., and Schneider, G. (1997) Identification of catalytically important residues in yeast transketolase. *Biochemistry* **36,** 15643–15649.