Molecular Biology Today 2001. 2(3): 61-66.

PCR Amplification of 1-Deoxy-D-Xylulose 5-Phosphate Synthase (*dxs*) Genes from Different *Streptomyces* species: Evidence for the Existence of Two *dxs* Gene Families

Shu-Ming Li*, Emmanuel Wemakor and Lutz Heide

Pharmazeutisches Institut, Pharmazeutische Biologie, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 8, D-72076 Tübingen, Germany

Abstract

Degenerate oligonucleotide primers were designed for the PCR amplification of 1-deoxy-D-xylulose 5-phosphate (DXP) synthase genes from genomic DNA of different *Streptomyces* strains. Prominent PCR fragments of the expected size (650 bp) were detected with all of the strains tested, and an additional band at 540 bp was observed with genomic DNA of *Streptomyces spheroides*. All of the PCR products showed high homology to DXP synthase (*dxs*) genes in the database. In total, 15 different *dxs* fragments were cloned from the five *Streptomyces* strains *S. spheroides* NCIMB 11891, *S. hygroscopicus* NRRL 3418, *S. rishiriensis* DSM 40489, *S. coelicolor* A3(2) and *S. lividans* TK 24. 11 of the 13 sequences could be placed into two different groups, distinguished by highly conserved motifs. Each examined organism contained at least one gene of each of the two groups. Three and five *dxs* isogenes, respectively, could be amplified from *S. hygroscopicus* NRRL 3418 and *S. spheroides* NCIMB 11891, which produce antibiotics with isoprenoid moieties. This is the first report of the existence of several putative *dxs* isogenes within single organisms.

Introduction

Isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are the common precursors of isoprenoids found in all organisms, such as steroid hormones in mammals, carotenoids and other isoprenoids in plants, ubiquinones and/or menaquinones in bacteria, and membrane lipids such as sterols and hopanoids (Sacchettini and Poulter, 1997). It was previously believed that IPP is always synthesized by condensation of three molecules of acetyl coenzyme A *via* the mevalonate pathway. However, it has recently been found that in plants and bacteria IPP can also be synthesized *via* a mevalonate-independent pathway (Rohmer *et al.,* 1993; Rohmer *et al.,* 1996; Lichtenthaler, 1999; Rohmer, 1999). The actinomycetes, a group of Gram-positive bacteria, which produce a large variety of antibiotics, can use the mevalonate pathway, the mevalonate-independent pathway or both pathways for the biosynthesis of their terpenoids (Shin-ya *et al.,* 1990; Seto *et al.,* 1996; Seto *et al.,* 1998).

Up to now, five genes have been identified which encode different enzymes of the mevalonate-independent pathway (Lüttgen *et al.,* 2000 and references cited herein). The initial step of this pathway is the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) by condensation of pyruvate and glyceraldehyde 3-phosphate, catalyzed by DXP synthase. The gene encoding DXP synthase (*dxs*) has been cloned from different bacteria and plants, e.g. from *Escherichia coli* (Sprenger *et al.,* 1997; Lois *et al.,* 1998), *Mentha x piperita* (Lange *et al.,* 1998), *Capsicum annuum* L (Bouvier *et al.,* 1998), *Synechococcus leopoliensis* (Miller *et al.,* 1999), *Rhodobacter capsulatus* (Hahn *et al.,* 2001), as well as from the *Streptomyces* sp. strain CL190 (Kuzuyama *et al.,* 2000) which produces the hemiterpenoid secondary metabolite naphterpin.

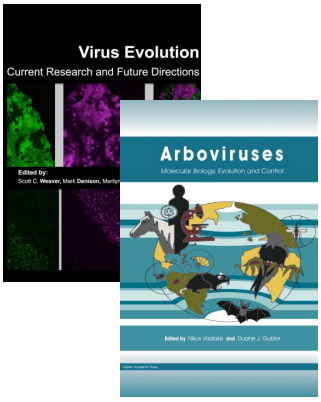
The coumarin antibiotics novobiocin and clorobiocin (=chlorobiocin) are produced by *Streptomyces spheroides* NCIMB 11891 and *S. hygroscopicus* NRRL 3418 (Berger and Batcho, 1978), respectively, and contain a dimethylallyl moiety. We have shown that this dimethylallyl moiety is derived from the mevalonate-independent pathway (Li *et al.,* 1998). Recently, we have cloned the biosynthetic gene cluster of novobiocin (Steffensky *et al.,* 2000). The cluster contains a total of 23 open reading frames and is flanked on one side by the novobiocin resistance gene *gyrB*^{R}, on the other side by an ABC transporter. However, neither the cluster nor the adjacent DNA regions contained genes involved in IPP formation. IPP for novobiocin biosynthesis may therefore be supplied by enzymes of the primary metabolism, or alternatively, specific genes for the biosynthesis of the isoprenoid moiety of novobiocin may exist, but be situated at a different locus of the genome. Screening of a genomic DNA library with one of the known genes of the mevalonate-independent pathway, e.g. *dxs*, as a probe may allow an investigation of the latter hypothesis.

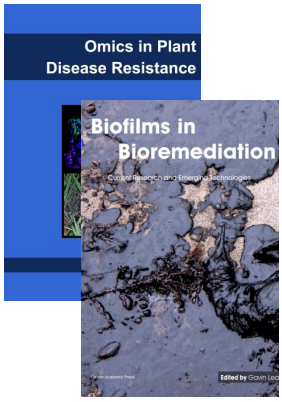
It has been reported that DXP is not only involved in the biosynthesis of IPP for the formation of isoprenoid

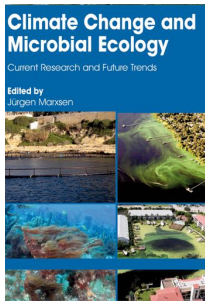
Further Reading

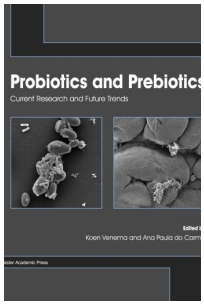
Caister Academic Press is a leading academic publisher of advanced texts in microbiology, molecular biology and medical research. Full details of all our publications at **caister.com**

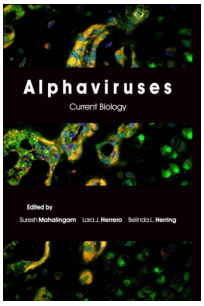
- **MALDI-TOF Mass Spectrometry in Microbiology**
  Edited by: M Kostrzewa, S Schubert (2016)
  www.caister.com/malditof
- ***Aspergillus* and *Penicillium* in the Post-genomic Era**
  Edited by: RP Vries, IB Gelber, MR Andersen (2016)
  www.caister.com/aspergillus2
- **The Bacteriocins: Current Knowledge and Future Prospects**
  Edited by: RL Dorit, SM Roy, MA Riley (2016)
  www.caister.com/bacteriocins
- **Omics in Plant Disease Resistance**
  Edited by: V Bhadauria (2016)
  www.caister.com/opdr
- **Acidophiles: Life in Extremely Acidic Environments**
  Edited by: R Quatrini, DB Johnson (2016)
  www.caister.com/acidophiles
- **Climate Change and Microbial Ecology: Current Research and Future Trends**
  Edited by: J Marxsen (2016)
  www.caister.com/climate
- **Biofilms in Bioremediation: Current Research and Emerging Technologies**
  Edited by: G Lear (2016)
  www.caister.com/biorem
- **Microalgae: Current Research and Applications**
  Edited by: MN Tsaloglou (2016)
  www.caister.com/microalgae
- **Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives**
  Edited by: H Shintani, A Sakudo (2016)
  www.caister.com/gasplasma
- **Virus Evolution: Current Research and Future Directions**
  Edited by: SC Weaver, M Denison, M Roossinck, et al. (2016)
  www.caister.com/virusevol
- **Arboviruses: Molecular Biology, Evolution and Control**
  Edited by: N Vasilakis, DJ Gubler (2016)
  www.caister.com/arbo
- ***Shigella*: Molecular and Cellular Biology**
  Edited by: WD Picking, WL Picking (2016)
  www.caister.com/shigella
- **Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment**
  Edited by: AM Romaní, H Guasch, MD Balaguer (2016)
  www.caister.com/aquaticbiofilms
- **Alphaviruses: Current Biology**
  Edited by: S Mahalingam, L Herrero, B Herring (2016)
  www.caister.com/alpha
- **Thermophilic Microorganisms**
  Edited by: F Li (2015)
  www.caister.com/thermophile

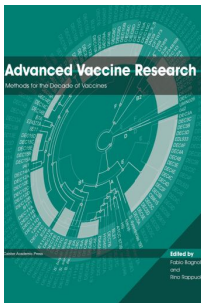


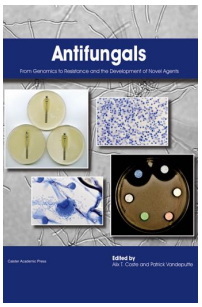












- **Flow Cytometry in Microbiology: Technology and Applications**
  Edited by: MG Wilkinson (2015)
  www.caister.com/flow
- **Probiotics and Prebiotics: Current Research and Future Trends**
  Edited by: K Venema, AP Carmo (2015)
  www.caister.com/probiotics
- **Epigenetics: Current Research and Emerging Trends**
  Edited by: BP Chadwick (2015)
  www.caister.com/epigenetics2015
- ***Corynebacterium glutamicum*: From Systems Biology to Biotechnological Applications**
  Edited by: A Burkovski (2015)
  www.caister.com/cory2
- **Advanced Vaccine Research Methods for the Decade of Vaccines**
  Edited by: F Bagnoli, R Rappuoli (2015)
  www.caister.com/vaccines
- **Antifungals: From Genomics to Resistance and the Development of Novel Agents**
  Edited by: AT Coste, P Vandeputte (2015)
  www.caister.com/antifungals
- **Bacteria-Plant Interactions: Advanced Research and Future Trends**
  Edited by: J Murillo, BA Vinatzer, RW Jackson, et al. (2015)
  www.caister.com/bacteria-plant
- ***Aeromonas***
  Edited by: J Graf (2015)
  www.caister.com/aeromonas
- **Antibiotics: Current Innovations and Future Trends**
  Edited by: S Sánchez, AL Demain (2015)
  www.caister.com/antibiotics
- ***Leishmania*: Current Biology and Control**
  Edited by: S Adak, R Datta (2015)
  www.caister.com/leish2
- ***Acanthamoeba*: Biology and Pathogenesis (2nd edition)**
  Author: NA Khan (2015)
  www.caister.com/acanthamoeba2
- **Microarrays: Current Technology, Innovations and Applications**
  Edited by: Z He (2014)
  www.caister.com/microarrays2
- **Metagenomics of the Microbial Nitrogen Cycle: Theory, Methods and Applications**
  Edited by: D Marco (2014)
  www.caister.com/n2

Order from caister.com/order

primary and secondary metabolites, but also in the biosynthesis of thiamin (vitamin B_1) (Thérisod *et al.,* 1981; Begley 1996) and pyridoxol phosphate (vitamin B_6) (Cane *et al.,* 1999; Laber *et al.,* 1999; Tazoe *et al.,* 2000) in bacteria. Therefore, several DXP synthase genes may exist for different biosynthetic pathways in a single *Streptomyces* strain. For studies of the function of these enzymes, it would be useful to clone the encoding *dxs* genes.

We now report a convenient method for the PCR amplification of *dxs* fragments from different *Streptomyces* strains, and the identification of several putative *dxs* isogenes from each of the examined strains.

Results

Design of Oligonucleotide Primers and PCR Amplification of 1-Deoxy-D-Xylulose 5-Phosphate Synthase Genes

Two conserved regions of *dxs* from 9 different bacteria (Figure 1) were selected to design the PCR primers under consideration of the codon usage for *Streptomyces* (Wright and Bibb, 1992). The forward primer (5´- GGS ATG GCS TGG GAR GCS CTS AAC; R = G or A; S = G or C) was derived from the amino acid sequence Gly Met Ala Trp Glu Ala Leu Asn. This motif is part of the putative thiamin diphosphate binding site of DXP synthases (Sprenger *et al.,* 1997; Lois *et al.,* 1998). The amino acid sequence Asp Val Gly(Ala) Ile Ala Glu Gln His (Figure 1) served for construction of the reverse primer (5´- GTG CTG YTC SGC GAT SSC SAC GTC; R = G or A; S = G or C; Y = C or T). The expected size of the PCR products obtained with these two primers is between 640 and 660 bp.

Genomic DNA from five *Streptomyces* strains was used for PCR amplification: *S. spheroides* NCIMB 11891 and *S. hygroscopicus* NRRL 3418 are the producers of the aminocoumarin antibiotics novobiocin and clorobiocin which contain a dimethylallyl moiety; *S. rishiriensis* DSM 40489 is the producer of the aminocoumarin antibiotic coumermycin A_1 which does not contain a terpenoid moiety (Berger and Batcho 1978); and *S. coelicolor* A3(2) and *S. lividans* TK 24 are the two best-examined *Streptomyces* strains, which are genetically very similar and which do not produce isoprenoid secondary metabolites (Kieser *et al.,* 2000; p. 426). The genome of *S. coelicolor* A3(2) has been almost completely sequenced (*Streptomyces coelicolor* genome project: http://www.sanger.ac.uk/Projects/S.coelicolor).

Prominent bands at about 650 bp were observed on agarose gels after PCR amplification with genomic DNA from all of the tested strains. With genomic DNA from *S. spheroides*, an additional band at about 540 bp was observed. The formation of these PCR products was dependent on the presence of genomic DNA and of both primers (data not shown).

Identification and Characterization of the Cloned *dxs* Fragments

The PCR products were cloned into the *Sma*I site of pBluescript SK(-) and the inserts were sequenced on both strands with standard M13 primers. All of 28 clones sequenced revealed very high similarity to the 1-deoxy-D-xylulose 5-phosphate synthases in the database. In total, the 28 clones represented 13 different genes: four from the novobiocin producer *S. spheroides*, three from the clorobiocin producer *S. hygroscopicus,* and two each from *S. coelicolor*, *S. lividans* and *S. rishiriensis*. As shown in Figures 2 and 3, the deduced amino acid sequences of these genes showed very high homology with each other (approximately 70 % identity at the amino acid level within 11 of the 13 genes). Especially, the first 50 amino acids of the examined sequences are very strongly conserved (>94 % identity within 11 of the 13 genes). The 540 bp fragment *dxsD*-Ss from *S. spheroides* showed a deletion of 38 amino acids from position 48 to 86, and a smaller deletion from position 148-151.

Phylogenetic analysis (Figure 3) of the PCR products revealed that, except *dxsD*-Ss and *dxsC*-Sh, the genes identified in this study can be placed into two groups. The distinction between these two groups is also obvious from the sequence alignment in Figure 2, most notably by the highly conserved motifs in position 152-161 (KIHPDTGLPI in group A and V(A)MDPLTCA(E)PL in group B).

At present, 98 % of the genome of S. *coelicolor* A3(2) have been sequenced, and the database contains two genes from this organism with homologies to DXP synthase. Indeed, our PCR method led to the amplification of fragments of both these genes: *dxsA*-Sc (656 bp) was identical (except for one base, and minor differences caused by wobbles in the backward primer) to an ORF sequenced on two overlapping cosmids in the database, SC1C3.01 and SC7B7.10 (AL023702 and AL009199, respectively), and *dxsB*-Sc (659 bp) was completely identical to the ORF SC6A5.17 (bp 17123-17781 of AL049485). *dxsA*-Sc and *dxsB*-Sc showed 68 % identity with each other on the amino acid level. Two corresponding genes were amplified from *S. lividans* TK 24, which is genetically very similar to *S. coelicolor*.

Since the *dxs* isogenes show very high homology, it may be expected that homologous recombination events can occur between these sequences in the bacterial genomes. Indeed, two of the 28 clones obtained in this study represented sequences which were likely to have originated from such recombination events: the fragment *dxsAxC*-Ss from *S. spheroides* NCIMB 11891 was completely identical to *dxsA*-Ss in the region from bp 1-449, and completely identical to *dxsC*-Ss in the region from bp 450-660, except for differences

caused by wobbles in the primer sequence. Likewise the fragment *dxsBxA*-Sc from *S. coelicolor* A3(2) was identical (except for 1 bp) to *dxsB*-Sc in the region from bp 1-367, and to *dxsA*-Sc from bp 368-660. Since these two sequences apparently did not represent independent genes, they were not included in Figures 2 and 3. They have been deposited in the GenBank database under accession number AF283711 (*dxsBxA*-Sc) and AF283721 (*dxsAxC*-Ss).

Discussion

With two degenerate primers derived from conserved regions of *dxs* genes from different bacteria, we have amplified a total of 13 putative *dxs* fragments using genomic DNA from five different *Streptomyces* strains.

The described method proved to be specific to putative *dxs* sequences. All of the 28 amplified PCR products showed very high homology to 1-deoxy-D-xylulose 5-phosphate synthase genes. Two to four different *dxs* fragments were cloned from each of the tested strains. The method is suitable for the amplification of probes for hybridization; the size of the PCR products (about 650 bp) is convenient to be subcloned and to be used as a probe for Southern hybridization.

The alignment (Figure 2) clearly showed that 11 of the 13 amplified genes could be placed into two groups (group A and group B), distinguished by motifs which are highly conserved within each group. Noteworthy, each of the examined *Streptomyces* strains contained at least one gene of group A and one gene of group B. The very high identity between these 11 genes and the functionally identified DXP synthase from *Streptomyces* sp. CL190 (Kuzuyama *et al.,* 2000) (>70 % on the amino acid level) makes it likely that all these genes encode DXP synthases. Of the two *S. coelicolor dxs* genes contained in the database, *dxsA*-Sc (= SC1C3.01/SC7B7.10) belongs to group A, and *dxsB*-Sc (= SC6A5.17) to group B. *dxsB*-Sc is situated in the *S. coelicolor* genome within an hopanoid/isoprenoid biosynthetic gene cluster, in the vicinity of a polyprenyl synthase, a squalen (or phytoene) synthase, a squalen (or phytoene) dehydrogenase and a squalene cyclase (Poralla *et al.,* 2000). This suggests that *dxsB*-Sc may be involved in IPP biosynthesis, and the same may be true for the other group B genes identified in our study. The genes of group A may represent biosynthetic genes engaged in thiamin and/or pyridoxol biosynthesis in *Streptomyces*. *dxs* genes from other organisms (e.g. *Mycobacterium tuberculosis*, *E. coli*, *Synechococcus leopoliensis* and others) could not be placed into one of the above groups and will probably deal with both biosynthetic pathways.

More than two *dxs* genes were found in *S. hygroscopicus* NRRL 3418 (3 genes) and *S. spheroides* NCIMB 11891 (4 genes). These organisms are the producers of clorobiocin and novobiocin, which contain an isoprenoid moiety formed *via* DXP. Therefore the identified, additional *dxs* isogenes may be involved in the biosynthesis of novobiocin and clorobiocin. However, some of the identified genes may also be non-functional, at least under the present culture conditions. Streptomycetes have a comparatively large genome (approximately 8000 kb) and quite commonly they contain gene clusters which are not functionally expressed (Hopwood, 1999 and references cited herein).

Streptomyces sp. CL190 (syn.: *S. aeriouvifer*) produces, besides the ubiquitous menaquinones, the hemiterpenoid secondary metabolite naphterpin (Seto *et al.,* 1996). Kuzuyama *et al.,* (Kuzuyama *et al.,* 2000) have amplified, cloned and expressed a single *dxs* gene from this organism. This gene clearly belongs to group B (Figure 2). It may be interesting to examine whether additional *dxs* genes can be identified from this organism, using our PCR method.

The present study describes, for the first time, the existence of several putative *dxs* isogenes in a single organism, and we developed a convenient method to amplify these genes from different *Streptomyces* strains. Given the importance of DXP synthase for the newly discovered isoprenoid biosynthetic pathway (which may prove an important target for new antibiotics and herbicides [Rohmer, 1998]), and for thiamin and pyridoxol biosynthesis, this PCR method may be a valuable tool for the future investigation of these pathways, enabling the cloning of several dxs isogenes from *Streptomyces* strains.

Experimental Procedures

Bacterial Strains, Plasmids and Culture Conditions

Plasmid vector pBluescript SK(-) was purchased from Stratagene (Heidelberg, Germany) and used for cloning of the PCR fragments. *Escherichia coli* XL1 Blue MRF' (Stratagene, Heidelberg, Germany) was used for the preparation of recombinant plasmids and grown in liquid Luria-Bertani (LB) medium or on solid LB medium (1.5 % agar) at 37°C (Sambrook *et al.,* 1989). Carbenicillin (50 µg/ml) was used for selection of recombinant strains.

S. hygroscopicus NRRL 3418 was from the Agricultural Research Service Culture Collection (Peoria, Illinois, USA). *S. rishiriensis* DSM 40489 was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). *S. spheroides* NCIMB 11891, *S. lividans* TK 24 and *S. coelicolor* A3(2) were obtained from E. Cundliffe (Leicester, UK) and D.A. Hopwood (Norwich, UK), respectively.

For preparation of genomic DNA, the *Streptomyces* strains were grown at 28°C and 175 rpm for 2-4 days in baffled shake flasks in YMG medium containing 1.0 % malt extract, 0.4 % yeast extract, 0.4 % glucose, and 1.0 mM $CaCl_2$ (pH 7.3) (Steffensky *et al.,* 2000).

DNA Isolation, Manipulation and Cloning

The genomic DNA of *Streptomyces* strains was isolated according to the method described by Kieser *et al.,* (Kieser *et al.,* 2000).

Isolation of PCR fragments from agarose gels for cloning was carried out with the Qiagen QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany).

Isolation of plasmids for sequencing was carried out with ion exchange columns (Nucleobond AX Kits, Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol.

Polymerase Chain Reaction (PCR) and Cloning of PCR Products

PCR was performed on a GeneAmp PCR System 9700 (Perkin-Elmer, Weiterstadt, Germany). The PCR reaction mixture (100 µl total volume) contained 0.5 µM of each primer (synthesized by Applied Biosystems, Weiterstadt, Germany), 500 ng genomic DNA of *Streptomyces*, 0.2 mM of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 5 % DMSO and 2.5 units of Taq DNA polymerase (Promega, Madison, USA). After denaturation at 96°C for 5 min, 25 cycles were carried out with 1.5 min at 95°C, 1.5 min at 65°C and 2.0 min at 72°C, and the final extension was performed at 72°C for 10 min. The PCR products were isolated from agarose gel and cloned blunt-end into the *Sma*I site of pBluescript SK(-) using the SureCloning Kit (Pharmacia, Freiburg, Germany).

DNA Sequencing and Computer-Assisted Sequence Analysis

DNA sequencing was performed by the dideoxynucleotide chain termination method using M13 primers on a LI-COR automatic sequencer (MWG-Biotech, Ebersberg, Germany).

The DNASIS software package (version 2.1, 1995; Hitachi Software Engineering, San Bruno, CA, USA) was used for sequence analysis. Amino acid sequence homology searches were carried out in the GenBank database using the BLAST programme (release 2.0).

Acknowledgments

This work was financially supported by the Deutsche Forschungsgemeinschaft (to L. H. and S.-M. L.)

References

Begley, T. P. 1996. The biosynthesis and degradation of thiamin (vitamin B_1). Nat. Prod. Rep. 13:177-185.

Berger, J., and Batcho, A. D. 1978. Coumarin-glycoside antibiotics. J. Chromatogr. Libr. 15:101-158.

Bouvier, F., d'Harlingue, A., Suire, C., Backhaus, R. A., and Camara, B. 1998. Dedicated roles of plastid transketolases during the early onset of isoprenoid biogenesis in pepper fruits. Plant Physiol. 117:1423-1431.

Cane, D. E., Du, S., Robinson, J. K., Hsiung, Y., and Spenser, I. D. 1999. Biosynthesis of vitamin B_6: enzymatic conversion of 1-deoxy-D-xylulose-5-phosphate to pyridoxol phosphate. J. Am. Chem. Soc. 121:7722-7723.

Hahn, F. M., Eubamks, L. M., Testa, C. A., Blagg, B. S. J., Baker, J. A., and Poulter, C. D. 2001. 1-Deoxy-D-xylulose 5-phosphate synthase, the gene product of open reading frame (ORF) 2816 and 2895 in *Rhodobacter capsulatus*. J. Bacteriol. 183:1-11.

Hopwood, D.A. 1999. Forty years of genetics with *Streptomyces*: from *in vivo* through *in vitro* to *in silico*. Microbiology 145:2183-2202.

Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., and Hopwood, D. A 2000. Practical *Streptomyces* genetics. The John Innes Foundation, Norwich, England.

Kuzuyama, T., Takagi, M., Takahashi, S., and Seto, H. 2000. Cloning and characterization of 1-deoxy-D-xylulose 5-phosphate synthase from *Streptomyces* sp. strain CL190, which uses both the mevalonate and nonmevalonate pathways for isopentenyl diphosphate biosynthesis. J. Bacteriol. 182:891-897.

Laber, B., Maurer, W., Scharf, S., Stepusin, K., and Schmidt, F. S. 1999. Vitamin B_6 biosynthesis: formation of pyridoxine 5´-phosphate from 4-(phosphohydroxy)-L-threonine and 1-deoxy-D-xylulose-5-phosphate by PdxA and PdxJ protein. FEBS Lett. 449:45-48.

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.

Li, S.-M., Hennig, S., and Heide, L. 1998. Biosynthesis of the dimethylallyl moiety of novobiocin *via* a non-mevalonate pathway. Tetrahedron Lett. 39:2717-2720.

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.

Lois, L. M., Campos, N., Rosa-Putra, S., Danielsen, K., Rohmer, and M., 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, thiamine, and pyridoxol biosynthesis. Proc. Natl. Acad. Sci. USA 95:2105-2110.

Lüttgen, H., Rohdich, F., Herz, F., 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-2C-methyl-D-erythritol. Proc. Natl. Acad. Sci. USA 97:1062-1067.

Miller, B., Heuser, T., and Zimmer W. 1999. A *Synechococcus leopoliensis* SAUG 1402-1 operon harboring the 1-deoxyxylulose 5-phosphate synthase gene and two additional open reading frames is functionally involved in the dimethylallyl diphosphate synthesis. FEBS Lett. 460:485-490.

Poralla, B., Muth, G. and Härtner, T. 2000. Hopanoids are formed during transition from substrate to aerial hyphae in *Streptomyces coelicolor* A3(2). FEMS Microbiol. Lett. 189:93-95.

Rohmer, M. 1998. Isoprenoid biosynthesis *via* the mevalonate-independent route, a novel target for antibacterial drugs? Drug Res. 50:136-154.

Rohmer, M. 1999. A mevalonate-independent route to isopentenyl diphosphate. In: Cane DE (ed) Comprehensive natural products chemistry. Isoprenoids including carotenoids and steroids. Vol 2, Elsevier: Oxford, U.K, pp 45-67.

Rohmer, M., Knani, M., Simonin, P., Sutter, B., and Sahm, H. 1993. Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem. J. 295:517524.

Rohmer, M., Seemann, M., Horbach, S., Bringer-Meyer, S., and Sahm, 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.

Sacchettini, J. C., and Poulter, C. D. 1997. Creating isoprenoid diversity. Science 277:1788-1789.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989 Molecular cloning: a laboratory manual 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Seto, H., Watanabe, H., and Furihata, K. 1996. Simultaneous operation of the mevalonate and non-mevalonate pathways in the biosynthesis of isopentenyl diphosphate in *Streptomyces aeriouvifer.* Tetrahedron Lett. 37:7979-7982.

Seto, H., Orihara, N., and Furihata, K. 1998. Studies on the biosynthesis of terpenoids produced by actinomycetes. Part 4. Formation of BE-40644 by the mevalonate and nonmevalonate pathways. Tetrahedron Lett. 39:9497-9500.

Shin-ya, K., Furihata, K., Hayakawa, Y., and Seto, H. 1990. Biosynthetic studies of naphterpin, a terpenoid metabolite of *Streptomyces*. Tetrahedron Lett. 31:6025-6026.

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.

Steffensky, M., Mühlenweg, A., Wang, Z.-X., Li, S.-M., and Heide, L. 2000. Identification of the novobiocin biosynthetic gene cluster of *Streptomyces spheroides* NCIB 11891. Antimicrob. Agents Chemother. 44:1214-1222.

Tazoe, M., Ichikawa, K., Hoshino, T. 2000. Biosynthesis of vitamin B_6 in *Rhizobium*. J. Biol. Chem. 275:11300-11305.

Thérisod, M., Fischer, J. C., and Estramareix, B. 1981. The origin of the carbon chain in the thiazole moiety of thiamine in *Escherichia coli*: incorporation of deuterated 1-deoxy-D-threo-2-pentulose. Biochem. Biophys.

Res. Commun. 98:374-379.

Wright, F., and Bibb, M. J. 1992. Codon usage in the G+C-rich *Streptomyces* genome. Gene 113:55-65.

	position (AA)	forward primer ⟶	position (AA)	reverse primer ⟵
Streptomyces sp. strain CL190	150	**GMAWEALN**	362	**DVGIAEQH**
Streptomyces coelicolor	150	**GMAWEALN**	362	**DVGIAEQH**
Mycobacterium tuberculosis	150	**GMCWEALN**	360	**DVGIAEQH**
Rhodobacter capsulatus	158	**GMAYEALN**	367	**DVGIAEQH**
Bacillus subtilis	150	**GMALEALN**	362	**DVGIAEQH**
Synechococcus PCC6301	150	**GMALEAIN**	365	**DVGIAEQH**
Haemophilus influenzae	158	**GMAFEALN**	366	**DVAIAEQH**
Escherichia coli	158	**GMAFEAMN**	365	**DVAIAEQH**
Helicobacter pylori J99	148	**GIFYEALN**	355	**DVAIAEQH**

Figure 1. Homologous regions in the amino acid sequences of *dxs* from *Streptomyces* sp. strain CL190 (Accession number AB026631), *Streptomyces coelicolor* (AL049485/SC6A5.17), *Mycobacterium tuberculosis* (O07184), *Rhodobacter capsulatus* (P26242), *Bacillus subtilis* (P54523), *Synechococcus* PCC6301 (CAB60078.1), *Haemophilus influenzae* (P45205), *Escherichia coli* (P77488) and *Helicobacter pylori* J99 (AE001468). The amino acid sequences GMAWEALN and DVG(A)IAEQH were used to design degenerate oligonucleotide primers for PCR amplification. The expected size of the PCR products is from 640 to 660 bp.

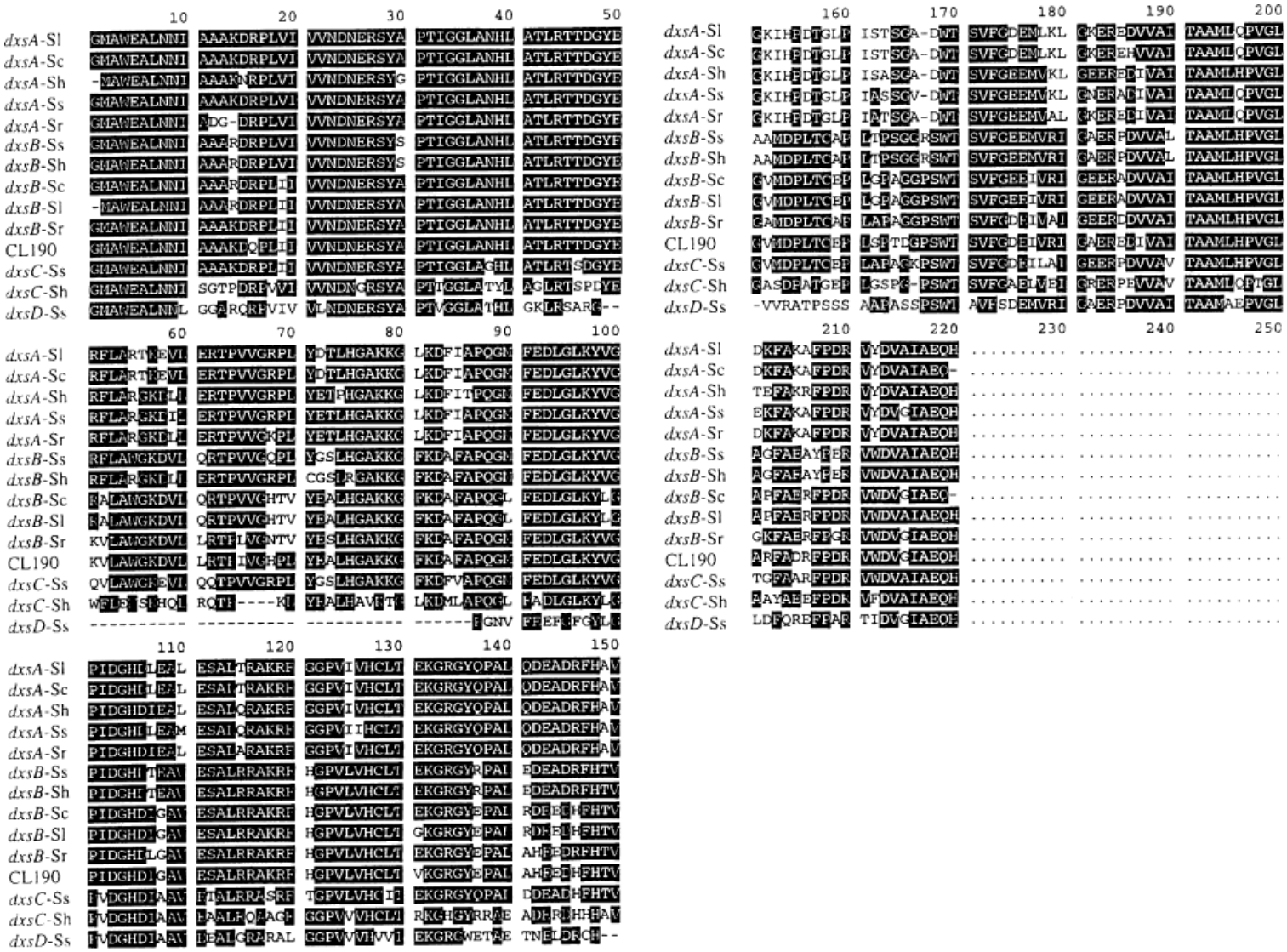


Figure 2. Alignment of the deduced amino acid sequences of the cloned 1-deoxy-D-xylulose 5-phosphate synthase fragments obtained by PCR using degenerate primers and genomic DNA. The nucleotide sequences are deposited in GenBank under the accession number indicated in parenthesis after the names; from *S. coelicolor*: *dxsA*-Sc (AF283709) and *dxsB*-Sc (AF283710); from *S. hygroscopicus*: *dxsA*-Sh (AF283712), *dxsB*-Sh (AF283713) and *dxsC*-Sh (AF283714); from *S. lividans*: *dxsA*-Sl (AF283715) and *dxsB*-Sl (AF283716): from *S. rishiriensis*: *dxsA*-Sr (AF283722) and *dxsB*-Sr (AF283723); from *S. spheroides*: *dxsA*-Ss (AF283717), *dxsB*-Ss (AF283718), *dxsC*-Ss (AF283719) and *dxsD*-Ss (AF283720). CL190 represents amino acids 150-369 of a DXP synthase which has been functionally identified from *Streptomyces*. sp. CL190 (Accesion number: AB026631) (Kuzuyama *et al.*, 2000).

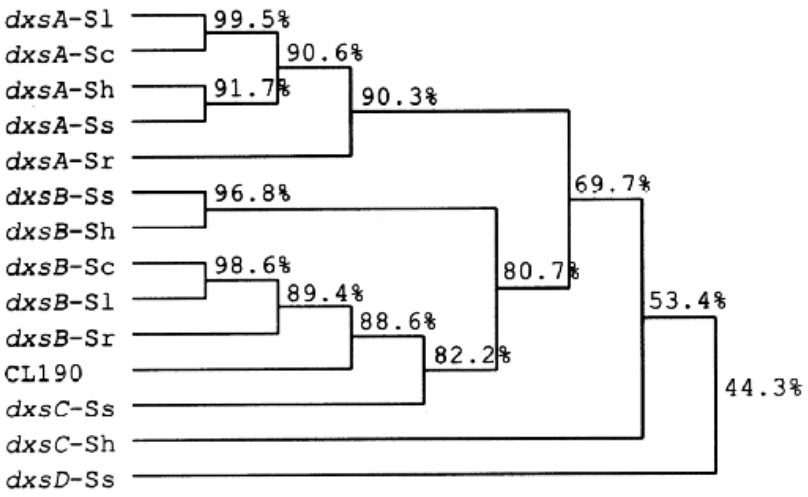


Figure 3. Phylogenetic tree based on sequence homologies. The amino acid sequences are described in the legend of Figure 2. The phylogeny was constructed using DNASIS for Windows, version 2 (Hitachi, San Bruno) scoring with a gap penalty of 5.0, a K-tuple of 2.0, a fixed gap penalty of 10.0 and a floating gap penalty of 10.0. The number of top diagonals and window size were both set at 5.