

Potential DNA slippage structures acquired during evolutionary divergence of *Acinetobacter calcoaceticus* chromosomal benABC and *Pseudomonas putida* TOL pWW0 plasmid xylXYZ, genes encoding benzoate dioxygenases

HARAYAMA, Shigeaki, et al.

Abstract

The xylXYZ DNA region is carried on the TOL pWW0 plasmid in *Pseudomonas putida* and encodes a benzoate dioxygenase with broad substrate specificity. The DNA sequence of the region is presented and compared with benABC, the chromosomal region encoding the benzoate dioxygenase of *Acinetobacter calcoaceticus*. Corresponding genes from the two biological sources share common ancestry: comparison of aligned XylX-BenA, XylY-BenB, and XylZ-BenC amino acid sequences revealed respective identities of 58.3, 61.3, and 53%. The aligned genes have diverged to assume G+C contents that differ by 14.0 to 14.9%. Usage of the unusual arginine codons AGA and AGG appears to have been selected in the *P. putida* xylX gene as it diverged from the ancestor it shared with *A. calcoaceticus* benA. Homologous *A. calcoaceticus* and *P. putida* genes exhibit different patterns of DNA sequence repetition, and analysis of one such pattern suggests that mutations creating different DNA slippage structures made a significant contribution to the evolutionary divergence of xylX.

Reference

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Potential DNA Slippage Structures Acquired during Evolutionary Divergence of *Acinetobacter calcoaceticus* Chromosomal *benABC* and *Pseudomonas putida* TOL pWW0 Plasmid *xylXYZ*, Genes Encoding Benzoate Dioxygenases

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The *xylXYZ* DNA region is carried on the TOL pWW0 plasmid in *Pseudomonas putida* and encodes a benzoate dioxygenase with broad substrate specificity. The DNA sequence of the region is presented and compared with *benABC*, the chromosomal region encoding the benzoate dioxygenase of *Acinetobacter calcoaceticus*. Corresponding genes from the two biological sources share common ancestry; comparison of aligned XylX-BenA, XylY-BenB, and XylZ-BenC amino acid sequences revealed respective identities of 58.3, 61.3, and 53%. The aligned genes have diverged to assume G+C contents that differ by 14.0 to 14.9%. Usage of the unusual arginine codons AGA and AGG appears to have been selected in the *P. putida* *xylX* gene as it diverged from the ancestor it shared with *A. calcoaceticus* *benA*. Homologous *A. calcoaceticus* and *P. putida* genes exhibit different patterns of DNA sequence repetition, and analysis of one such pattern suggests that mutations creating different DNA slippage structures made a significant contribution to the evolutionary divergence of *xylX*.

Benzoate dioxygenase (30) catalyzes the first of two step reactions that give rise to catechol from benzoate. Many bacterial species, exemplified by *Acinetobacter calcoaceticus*, carry chromosomal genes for enzymes that convert catechol to citric acid cycle intermediates via β-ketoadipate (20, 21, 23, 31). These enzymes do not act effectively upon methylsubstituted substrates. Methylcatechols, formed from methylbenzoates by enzymes with relatively broad substrate specificity, are utilized by a different metabolic pathway (2). Genes for this pathway frequently are carried on plasmids such as the TOL pWW0 plasmid from *Pseudomonas putida* (8, 36).

Isofunctional enzymes for dissimilation of aromatic compounds in *A. calcoaceticus* and *P. putida* generally exhibit close evolutionary ancestry reflected in identities of amino acid sequence close to or exceeding 50% (9, 23, 38, 39, 41). In contrast, genes for such isofunctional enzymes have diverged substantially, as indicated by differences in G+C content of about 15% (9, 20, 27). Such differences in G+C content may be attributed in part to directional pressure exerted by mutations within divergent cell lines (32). In addition, selection for cognate tRNAs characteristic of the host (1, 11) appears to have contributed to divergence of the G+C content in DNA because genes from either *A. calcoaceticus* or *P. putida* tend to possess a pattern of codon usage that is similar to that of other genes from closely related organisms (27, 35).

A question that remains is the nature of mutations that gave rise to divergent DNA sequences encoding homologous genes in the two bacterial species. Comparison of amino acid sequences suggested that gene conversion events causing substitution of oligonucleotide sequences within (29) and

among (40) coevolving genes created sequence repetitions that were a major source of genetic divergence. The data were puzzling because they implied that DNA sequences encoding dipeptides and tripeptides were shuffled within genes during their evolutionary divergence. The evidence suggested that sequence exchange between misaligned DNA strands might have been a source of the shuffling mutations (25, 29), but in the absence of DNA sequences for the divergent genes, it was not possible to suggest specific mechanisms that created and maintained the divergent sequences.

Analysis of a range of biological systems has suggested that sequence exchange among slipped DNA strands caused mutations that have been a significant source of evolutionary divergence (17, 34). The interaction of slipped DNA strands during mutation has been documented by demonstration that sequence-directed mutations create both deletions (3–6) and repetitions of DNA sequence. Comparison of DNA sequences for oxygenative enzymes from *A. calcoaceticus* and *P. putida* suggested that slippage structures formed between misaligned DNA strands were formed by mutation and are maintained by mismatch repair during evolution (9, 21).

Elsewhere, we have reported the DNA sequences of the chromosomal *A. calcoaceticus* *benABC* genes, which encode a benzoate dioxygenase with relatively narrow substrate specificity; the enzymes encoded by these genes proved to be members of widely extended families of proteins associated with oxygenative processes and electron transport (22). Here we present the DNA sequences of *P. putida* TOL plasmid DNA containing the *xylXYZ* genes which encode a benzoate dioxygenase with broad substrate specificity. Comparison of the *benABC* and *xylXYZ* genes demonstrates their overall homology, reveals different patterns of sequence repetition that were acquired during their divergence, and suggests specific, potentially interactive,

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DNA slippage structures that were acquired during the evolution of *xylZ*.

MATERIALS AND METHODS

Sources of DNA for sequencing were pPL392 (7) and subclones derived from it (8). DNA fragments generated by cleavage with different restriction enzymes were subcloned into mp18 or mp19 and propagated in *Escherichia coli* JM101 (37). DNA sequencing with M13 derivatives as templates was done with a kit from Pharmacia. Overlapping sequences from both strands were determined. Sequence analysis was performed with PC/Gene (Intelligenetics) and Microgenie (Beckman) software packages.

Nucleotide sequence accession number. The *xyl* DNA sequence presented in this report has been deposited with GenBank under accession number M64747.

RESULTS

Overall sequence comparisons. Figure 1 presents DNA sequences containing the *P. putida* *xylXYZ* genes and the *A. calcoaceticus* *benABC* genes accompanied by the aligned amino acid sequences of the gene products. Three comparisons, XylX-BenA, XylY-BenB, and XylZ-BenC, revealed respective amino acid sequence identities of 58.3, 61.3, and 53%. Regions of amino acid sequence similarity were distributed fairly evenly through the compared regions, and thus it appears likely that the *xylXYZ* and *benABC* genes were derived from a common ancestral DNA fragment.

DNA sequences lying between the aligned genes were not subjected to selection at the level of protein, and these regions have diverged. Whereas the *benA* and *benB* genes overlap by a single nucleotide, the corresponding *xylX* and *xylY* genes do not overlap (Fig. 1). The distance between *benB* and *benC* is 66 nucleotides longer than the distance between *xylY* and *xylZ*. The *ben* and *xyl* sequences show no similarity in this region, but it would be incorrect to conclude that this segment of DNA escaped selection for structure because the *ben* sequence contains an inverted repetition 10 bp in length (Fig. 1).

G+C content and codon usage. Despite their similarity at the level of translation, the *P. putida* and *A. calcoaceticus* genes differ substantially in G+C content. Whereas the respective G+C contents of the *P. putida* *xylX*, *xylY*, and *xylZ* genes are 59.3, 56.1, and 61.3%, the G+C contents of the corresponding *A. calcoaceticus* *benA*, *benB*, and *benC* genes are 45.0, 41.2, and 47.3%, respectively. Thus, the differences in the G+C content of the *xyl* genes range over 4.2%, and the differences in the G+C content of the *ben* genes range over 6.1%. Differences in the G+C content emerging from *xylX-benA*, *xylY-benB*, and *xylZ-benC* comparisons fall within the relatively narrow range between 14.0 and 14.9%.

Variation in the G+C content of genes from different organisms has been attributed to a combination of directional mutation pressure toward a defined G+C content (32) and selection for codon usage as determined by the relative pool levels of cognate tRNAs (1, 11). Comparison of codon usage in the *xyl* and *ben* genes showed that differences are found most frequently in the third nucleotide position, which allows codons with different G+C contents to encode identical amino acid residues. For example, 60 arginyl residues expressed by the *P. putida* *xylXYZ* genes are encoded with respective frequencies of 57 and 17% by CGC and CGT. Conversely, the 49 arginyl residues in the *A. calcoaceticus*

genes are encoded by CGC and CGT with relative frequencies of 22.4 and 59.2%, respectively.

DISCUSSION

Conservation of genes and divergence of intergenic sequences. Amino acid sequence comparisons provide strong evidence for common ancestry of the *xylXYZ* and *benABC* regions: identity of amino acid sequence in the aligned gene products (Fig. 1) exceeds 53%. Intergenic regions of DNA have not been subjected to selection at the level of protein function, and substantial divergence of DNA sequence occurred in these noncoding regions. The single-base overlap of *benA* and *benB* prompts speculation that their expression might be concerted by translational coupling (24). Whatever the merits of this proposal, it must be recognized that the putative control has not been conserved in the closely homologous *xylX* and *xylY* genes, which do not overlap (Fig. 1). Possible genetic or physiological significance might be ascribed to the 10-bp inverted repetition lying between *benB* and *benC* (Fig. 1), but selective forces favoring the inverted repetition were not conserved during divergence of *xylY* and *xylZ* between which the corresponding region of DNA is not present (Fig. 1).

Use of exceptional arginine codons in *xylX*. An unusual property of the *xylX* gene is its use of two AGA and three AGG codons for 5 of the 35 arginyl residues. These codons are employed rarely by structural genes from fluorescent *Pseudomonas* species (35). Nor are the five exceptional codons used by any of the 17 arginyl residues conserved in *xylX* during its divergence from *benA* (Fig. 1). Thus, the rare AGA and AGG codons represent 5 of 18 arginyl codons that were either acquired or maintained during divergence of *xylX* from the ancestor it shared with *benA*. It therefore is reasonable to conclude that the unusual arginyl codons in the *xylX* gene were selected. The basis for the selection is unknown. Remarkable enrichment of the exceptional arginyl codons is also found in other *xyl* genes from the TOL plasmid pWW0 (10, 12, 13, 19, 33). These observations raise the possibility that the *xyl* structural genes have been selected in an organism in which tRNA corresponding to AGA and AGG codons was relatively high.

Evolutionary acquisition of DNA slippage structures. Different patterns of DNA sequence repetition distinguish the *xyl* and *ben* regions. As discussed below, it appears that the sequence repetitions arose as a consequence of mutations that superimposed DNA sequences from different chromosomal regions during evolutionary divergence. The question that must be addressed is how to account for both the origin and the maintenance of complex patterns of DNA sequence repetition.

Acquired DNA sequence repetitions have been interpreted as components of three-dimensional structures formed by hybridization between slipped strands (25–29). According to this view, the genetic basis for the formation and the conservation of the DNA slippage structures is mismatch repair between the hybridizing regions (16, 18). The model predicts that DNA forced into single strandedness in one slippage structure may hybridize with a complementary DNA sequence in another region and thus may foster formation of an alternative slippage structure. Thus, interactive slippage structures could contribute to genetic continuity and, when disrupted, generate cascades of mutation through mismatch repair. Therefore, the model accounts for how rapid genetic divergence could occur and, once achieved, how it might be maintained.

	10	20	30	40	50	60	70	80	90	100	110	120	
xyl ben	TAACGACGCTGGTCGTCTCGTGCCTGCTGGTGCCTGCTGACCCCTGCCGTGCTCATCGGCCCTCGTGGACGGCCTGTCGCCGACATCGCGCTTCGCCGCGGAGC AAGCTTG 1												
xyl ben	CGAGTCGGGGTTCTATCACCGGCCAGGGCCAGGCCGATCCCCCTGCCGTACTGCCGTGGGTGACTTACCTGGCACTGCCGTGACGCCGCTGCTGATCTCGTCCCAG CTCTCAACACACGCCAACAAAATAGCGGAGATGCTAAGTCCATTAAAGACTCCATAGGTATTTTATAACAATAATGTTGAACCTTATAAACACATTCTTAAGGTATAA 10 20 30 40 50 60 70 80 90 100 110 120	130 140 150 160 170 180 190 200 210 220 230 240											
xyl ben	C CGCGCACTGCTCGCGTGGCGGTGTCATCGCTGCCGACGTTCAAGAAGTATCTAGGTGCCACGTTAATTGATCAGCAGTCCCTAACGGCTGCACTGTCAGTGTCCGGTTTGATAG ACAAGCAAGAACACAAGAAGCAGGGCTGACCCATTAAATGCTTCAATTGGAAAATTGAAAGCTGAAATGGATATTGTTCTGGGTTCTGCCGTTAAGTAA 130 140 150 160 170 180 190 200 210 220 230 240	250 260 270 280 290 300 310 320 330 340 350 360											
xylX benA	GGATAAGTCCAGCCTTGCAGAACGGATACAGAGTGCACAAAATGGCTATCTAGAAGGCCAACCGCTTCTAGGCTTATGCAACAGAACATAATAATGGAGTCAGTACCCATGACA CATTATGCGTTGCGTTTAAATGAATGTTGACTAAGCACACGGTTGCTCTGCCCTAGACAAGTTCTTATTTGGAATGTTGAGAAGGATATGCCACGTTCCCGTATT 250 260 270 280 290 300 310 320 330 340 350 360	370 380 390 400 410 420 430 440 450 460 470 480											
XylX BenA	NetThr MetProArgileProValle												
xylX benA	ATGCACCTGGGGCTCGACTATAGATAGTCTCGTGAAGAAGATGAGAACGAGGGCATCTACCGCTGCAAGGCCGAGATGTTACCCGACCCCTGGCTGTTGGATTAGAGATGAAACAC AATACTAGCCATCTTGACCGAATTGTAAGTCACTGCTGAGACAATACCGAACAGGTGAATTAAAGTTACATCGTTCTGTATTACAGATCAGGACTTTTGATCTGAATGAAATAC 370 380 390 400 410 420 430 440 450 460 470 480	490 500 510 520 530 540 550 560 570 580 590 600											
XylX	MetHisLeuGlyLeuAspTyrileAspSerLeuValGluGluAspGluAsnGluGlyileTyrArgCysLysArgGluMetPheThrAspProArglePheAspLeuGluMetLysHis	*	*	*	*	*	*	*	*	*	*	*	
BenA	AsnThrSerHisLeuAspArgileAspGluLeuLeuValAsnThrGluThrGlyGluPheLysLeuHisArgSerValPheThrAspGlnAlaLeuPheAspLeuGluMetLysTyr	*	*	*	*	*	*	*	*	*	*	*	
xylX benA	ATCTTGAGGCCACTGGATTATCTGGCCACAGGAGGCCAGATTCCCGAGAGAACGACTATTACACCACCGAGATGGCCGGCAGCCGATATTCAACACGCCAACAAAGATGGTAG ATTTCGAGGAAATTGGGTTATTGCGCTATGAAAGCCAGATTCCAACAAACAGCACTATTACACCCTATTGGAGACACCGATTGATGGCGCGTAATCCGAAACGGTGAA 490 500 510 520 530 540 550 560 570 580 590 600	610 620 630 640 650 660 670 680 690 700 710 720											
XylX	IlePheGluGlyAsnTrpIleTyrLeuAlaHisGluSerAspTyrTyrThrGlnMetGlyArgGlnProIlePheileThrArgAsnLysAspGlyGlu	*	*	*	*	*	*	*	*	*	*	*	
BenA	IlePheGluGlyAsnTrpValTyrLeuAlaHisGluSerGinileProAsnAsnAspTyrTyrThrTyrileGlyArgGinProIleLeuileAlaArgAsnProAsnGlyGlu	*	*	*	*	*	*	*	*	*	*	*	
xylX benA	CTGAATGCCCTCGCAATGCTGAGTCACCGCGGCCACGCTCTGCTGTTAGGAGTGGAAACAAAGCCACCCACCTGCTGTTCCACGGCTGGACCTTCAGCAATTGGCGAAG CTCAACGCCATGATTAAACGATGTTCACTEGTGGTGACAGCTGCTGGTATAAGCGTGGATAAAAGCCACATATACTTGCCATTTCATGGCTGGACCTTCATAACTCAGGAAA 610 620 630 640 650 660 670 680 690 700 710 720	730 740 750 760 770 780 790 800 810 820 830 840											
XylX	LeuAsnAlaPheValAlaAsnAlaCysSerHisArgGlyAlaThrLeuCysArgpheArgSerGlyAsnLysAlaThrHisThrCysSerPheHisGlyTrpThrPheSerAsnSerGlyLys	*	*	*	*	*	*	*	*	*	*	*	
BenA	LeuAsnAlaMetIleAsnAlaCysSerHisArgGlyAlaGlnLeuLeuGlyHisLysArgGlyAsnLysThrThrTyrCysProPheHisGlyTrpThrPheAsnAsnSerGlyLys	*	*	*	*	*	*	*	*	*	*	*	
xylX benA	CTGCTCAAGGTCAAAGACCCCCAACGGTGCCTGCTATCGGACACGCTTGCAGTGTGACGGCTGCCACGCCCTGAAAGAATTGCGCTTTCGCTCCACCGGGATTCTATTGGCGAC ATGTTGAAGGTGAAAGATCCAAGCGATGCTGGTTATTGAGATTGTTAACTGAGACGGTCCCACACTTAAAGGTTGGCGCTTTGAAAGTTAAAGGTTTATTGGCGAGT 730 740 750 760 770 780 790 800 810 820 830 840	850 860 870 880 890 900 910 920 930 940 950 960											
XylX	LeuLeuLysValLysAspProLysGlyAlaGlyTyrProAspSerPheAspCysAspGlySerHisAspLeuLysValAlaArgPheAlaSerTyrArgGlyLeuPheGlySer	*	*	*	*	*	*	*	*	*	*	*	
BenA	LeuLeuLysValLysAspProSerAspAlaGlyTyrSerAspCysPheAsnGlnAspGlySerHisAspLeuLysValAlaArgPheGluSerTyrLysGlyLeuPheGlySer	*	*	*	*	*	*	*	*	*	*	*	
xylX benA	CTGCCGAGGACGCTGCCCGTTGGAAAGTTCTCGCGAGTCAGGAGGTATCGACATGGCTGACCGAGTGGCTGAGGCTGGAGATGGCTGCGCCGTTCCAGTACCTATGAT CTGAATCTGTAGATCCGTCAGTCAGAGTTTGGGGAAACCCACAAATTATGACATGATTGTCGGCAATCCGATCAGGGCTTGAAGTACTCGTGGTGTTCGACCTACCC 850 860 870 880 890 900 910 920 930 940 950 960	970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080											
XylX	LeuArgGluAspValAlaProLeuGluGluPheLeuGlyGluSerArglysValileAspMetValAspGlnSerProGluGlyLeuGluValLeuArgGlySerSerThrTyrVal	*	*	*	*	*	*	*	*	*	*	*	
BenA	LeuAsnProValAspProSerLeuGlnGluPheLeuGlyGluThrThrLysileileAspMetileValGlyGlnSerAspGlnGlyLeuGluValLeuArgGlyValSerThrTyrThr	*	*	*	*	*	*	*	*	*	*	*	
xylX benA	TACGAAGGAACTGGAAAGTGCAGGTCAGAACGGTCCGCCACGGCTACCCAGTCAGTACTGTTACTGAAACTACCCGCCACCCAGCAGCGCAAGCTGAGAGACGGGGCGATGAT TATGAAGGAAACTGGAAAGTTGACCCAGAAAACGGAGCAGATGGCTATCATGTTGGCGCTGACTGGAAACTATGCAAGCACCACCGCAGCATGTAAGAAAAACGGCAGGTGATACC 970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080	1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200											
XylX	TyrGluGlyAsnTrpLysValGlnValGluAsnGlyAlaAspGlyTyrHisValSerThrValHisTrpAsnTyrAlaAlaThrGlnGlnGlnArgLysLeuArgAspAlaGlyAspAsp	*	*	*	*	*	*	*	*	*	*	*	
BenA	TyrGluGlyAsnTrpLysLeuThrAlaGluAsnGlyAlaAspGlyTyrHisValSerAlaValHisTrpAsnTyrAlaAlaThrGlnHisArgLysGluLysGlnAlaGlyAspThr	*	*	*	*	*	*	*	*	*	*	*	
xylX benA	ATTCCGCGCATGACGCCAGTAGCTGGGGGGGGATGGCGCCGGTTCTACTCCTTGAACACGGCACCAGATGGCTGGGCACGCTGGGGTGAACCGGAAACCGCCCGCTGTCGCC ATTCCGCGCATGACGCCGGCTCGTGGGGAAACATGGTGGCGCTCATATGGATTGAAACATGGTCTGCTGACACATGGTAACTGGGTAATCCGAAAGCCGACAAACTTCC 1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200	1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320											
XylX	IleArgAlaMetThrAlaSerSerTrpGlyGlyAspGlyGlyPheTyrSerPheGluAsnGlyHisGlnMetValTrpAlaArgTrpGlyAspProLysAsnArgProLeuPheAla	*	*	*	*	*	*	*	*	*	*	*	
BenA	IleArgAlaMetSerAlaGlySerTrpGlyGlyLysHisGlyGlySerTyrGlyGlyPheGluHisGlyHisGlnLeuLeuTrpThrGlnTrpGlyAsnProGluAspArgProAsnPhePro	*	*	*	*	*	*	*	*	*	*	*	

FIG. 1. Aligned DNA sequences of *P. putida* *xylXYZ* and *A. calcoaceticus* *benABC*. Potential ribosome binding sites preceding each gene are underlined. Asterisks mark amino acid residues shared in the protein products of the aligned genes. Vertical lines flank asterisks marking arginyl residues conserved in XylX and BenA. Exclamation points mark the exceptional AGA and AGG arginyl codons that appear to have been selected in *xylX*. Arrows indicate a perfect 10-bp repetition in the DNA sequence lying between *benB* and *benC*.

	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430	1440
<u>xylX</u>	GAGCAGATCGCTAGCCAGCGAGTTGGTGAAGGCCGTCGGACTGGATGATCGCGTCTCCCGAACCTCTGCCTCACCGAACCTCACCGTGTGGACCAGTCGGCTCGACGTG											
<u>benA</u>	AAAGCAGCGAATACCGAAAATTCCGTGCTGCAATGATGATCGAACGCTCACGTAACCTGTGTTATCAGGATGATGGTACCTGATGGTCAAGTTCGGTCAAGT											
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320	
XylX	GluArgAspArgLeuAlaSerGluPheGlyGluAlaArgAlaAspTrpMetIleGlyValSerArgAsnLeuCysLeuTyrProAsnLeuTyrLeuMetAspGlnPheGlySerGlnLeu	*	*	*	*	*	*	*	*	*	*	*
BenA	LysAlaAlaGluTyrThrGluLysPheGlyAlaAlaMetSerLysTrpMetIleGluArgSerArgAsnLeuCysLeuTyrProAsnValTyrLeuMetAspGlnPheGlySerGlnIle	*	*	*	*	*	*	*	*	*	*	*
	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	
<u>xylX</u>	CGTATCACCCGTCGGCTGCGTGGATAGAACCGAAATCACCATCTACTGCATCGGCCAACGGCAAACGCC---AGGCCTGCCGCCGTCGGCTCAGTACAGGAGACTTCCTCAAT											
<u>benA</u>	CGTGTTCAGTCCAATTTCGGTCATAAAGCGAGTCACCATTAATGCGCTGAGTATGGCCTGAGCTGAGCAGCCGTCAGTATGCAAGGATTTCTTAAT											
1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430	1440	
XylX	ArgIleThrArgProLeuSerValAspArgThrGluIleThrIleTyrCysIleAlaProLysGlyGluThrPro---ArgArgAlaArgArgValArgGlnTyrGluAspPheAsn	*	*	*	*	*	*	*	*	*	*	*
BenA	ArgValLeuArgProIleSerValAsnLysThrGluValThrIleTyrCysIleAlaProValGlyGluAlaProGluAlaArgAlaArgGlnTyrGluAspPheAsn	*	*	*	*	*	*	*	*	*	*	*
	1560	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670
<u>xylX</u>	GTCAGCGCATGGCCACCCGGACCTGGAGGAATTCCGGCCCTGCCAGGAGGGCTTCGCCGGCGGGGGATG-----AACGACATGTCGGCGCAAACACTGGATCGAGGGG											
<u>benA</u>	GCATCTGGAATGGGACGCGACGATCTTGAGGAGTTCGCTCGCTGAGCTGAGTATGGAAACTGGACATGTGCCGGATCAAACATTGGATTATGGG											
1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560	
XylX	ValSerGlyMetAlaThrProAspAspLeuGluGluPheArgAlaCysGlnGluGlyPheAlaGlyGlyMet-----AsnAspMetSerArgGlyAlaLysHisTrpIleGluGly	*	*	*	*	*	*	*	*	*	*	*
BenA	AlaSerGlyMetAlaThrProAspAspLeuGluGluLeuProArgCysGlnAlaGlyIleGluLeuGluTrpAsnAspMetCysArgGlySerLysHisTrpIleTyrGly	*	*	*	*	*	*	*	*	*	*	*
	1680	1690	1700	1710	1720	1730	1740	1750	1760	1770	1780	1790
<u>xylX</u>	CCGACGAGGGCGGAAGGAGATCGATCTGCATCGGAAGCTGAGCGGTGTCGCGCTCGGAAGCGAAGGCCGTCGCTCATGCAGCACAGTACTGGCAACAGCAGATGATCAAGGGCGTC											
<u>benA</u>	CCAGATGATGCCGCTAAATGAATCGGATTAAACCGGCTATTAGTGGTATTAACGACTGAAGACGAAAGGCTGTGATTTGGCACAGCATCAACTACTGGCTCAAAGTATGAAAGCAAGCATT											
1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	
XylX	ProAspGluGlyAlaLysGluIleAspLeuHisProLysLeuSerGlyValArgSerGluAspGluGlyLeuPheValMetGlnHisLysTyrTrpGlnGlnGlnMetIleLysAlaVal	*	*	*	*	*	*	*	*	*	*	*
BenA	ProAspAspAlaAlaAsnGluIleGlyLeuLysProAlaIleSerGlyIleLysThrGluAspGluGlyLeuTyrLeuAlaGlnHisGlnTyrTrpLeuLysSerMetLysGlnAlaIle	*	*	*	*	*	*	*	*	*	*	*
	1800	1810	1820	1830								
<u>xylX</u>	AAGCGAACAGGATCGGCTGATCCATGCGGAGGGCGTGTAA-----ATGACTATCTCTACGAAGCCGTGCGCATTCTTACCGCGAACGACGCTACCTC											
<u>benA</u>	GCTGCGAAAAAGAATTTCGATCGCGTCAGGGAGAGAACGATGAATGCTACAGCACTTTAGACACCCTAGCATCGAACAGATTAGCCAGTTTTGTATAGCGAGGCCGTTTTTA											
1690	1700	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800	
XylX	LysArgGluGlnAspArgLeuIleHisAlaGluGlyVal	*	*	*	*	*	*	*	*	*	*	*
BenA	AlaAlaGluLysGluPheAlaSerArgGlnGlyGluAsnAla	*	*	*	*	*	*	*	*	*	*	*
XylY	MetThrIleSerTyrGluAlaValArgAspPheLeuTyrArgGluAlaArgTyrLeu	*	*	*	*	*	*	*	*	*	*	*
BenB	MetAsnAlaThrAlaLeuLeuAspThrIleSerIleGluGlnIleSerGlnPheLeuTyrSerGluAlaArgPheLeu	*	*	*	*	*	*	*	*	*	*	*
	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990	2000	2010
<u>xylY</u>	GACGACAAGCAGTGGGAAAGCTGGCTGGAAATGACGGCGCCGCACTTCTGGATGCCGCTGGAGCACGCCAACCTGACGAGGCCAGCTCGCTGATT											
<u>benB</u>	GATGATGAGCAATGGGATGACTGGCTGAATGTTAGCACCTCAAGCTCATTTGGATGCCGCTGGGAGGATAACGACAGCTACTGAAACCCCCAAACTGAGTTCCGCTGATT											
1810	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910	1920	
XylY	AspAspLysGlnTrpGluSerTrpLeuGluMetTyrAlaProAspAlaThrPheTrpMetProAlaTrpAspAspArgAspGlnLeuThrGluAspProGinSerGlnSerLeuIle	*	*	*	*	*	*	*	*	*	*	*
BenB	AspAspGluGlnTrpAspTrpLeuGluCysTyrAlaProGlnAlaSerPheTrpMetProAlaTrpAspAspAspGlnLeuThrGluAsnProGlnThrGluIleSerLeuIle	*	*	*	*	*	*	*	*	*	*	*
	2020	2030	2040	2050	2060	2070	2080	2090	2100	2110	2120	2130
<u>xylY</u>	TGGTACGGCAATCGCAGTGGCTAGAGGATCGGGTGTCCGCATAGACCGAGGGCTTCAGTGCACCATTCGGACACCCGACACATCAGCAATTGGAGTTGCTCGAG											
<u>benB</u>	TATTATCCAGATGCCAAGGCTGAAGATCGGATTTCGGGATAAAACCGGCTTCAGGCTACGGGACCATGGGACGATACGGTACGGCACACAATTAGCAATATCGAAGTTGAATCA											
1930	1940	1950	1960	1970	1980	1990	2000	2010	2020	2030	2040	
XylY	TrpTyrGlyAsnArgSerGlyLeuGluAspArgValPheArgIleLysThrGluArgSerSerAlaThrIleProAspThrArgThrSerHisAsnIleSerAsnLeuGluLeuGlu	*	*	*	*	*	*	*	*	*	*	*
BenB	TyrTyrProAspArgGlnGlyLeuGluAspArgValPheArgIleLysThrGluArgSerSerAlaThrMetProAspThrArgThrAlaHisAsnIleSerAsnIleGluValGluSer	*	*	*	*	*	*	*	*	*	*	*
	2140	2150	2160	2170	2180	2190	2200	2210	2220	2230	2240	2250
<u>xylY</u>	CAGTCGGATGGCGCTGTAAGCTGCGCTACAACGACCATGAAATTCTGGTACAAGACGGTGGACCACTTCTTGGACCAATTCTGCACTCTGCACACATGCGGAGACCCG											
<u>benB</u>	CGTATGGCCTTCAAAATCACAGTACGTTAACGCTAGTTCCGCTATAAAACAGTTACGCTATTGGCATGTCACGCTATGTAATCGATTGCAAGTTCAGGTGAACACCA											
2050	2060	2070	2080	2090	2100	2110	2120	2130	2140	2150	2160	
XylY	GlnSerAspGlyValCysLysLeuArgTyrAsnTrpHisThrMetAsnTyrLysThrValAspHisPheGlyThrAsnPheCysThrLeuAspThrCysGlyGluThrPro	*	*	*	*	*	*	*	*	*	*	*
BenB	ArgAspGlyLeuGlnIleThrValArgPheAsnTrpAsnThrLeuSerPheArgTyrLysAsnSerTyrSerTyrPheGlyMetSerArgTyrValIleAspPheSerGlyGluGlnPro	*	*	*	*	*	*	*	*	*	*	*
	2260	2270	2280	2290	2300	2310	2320					
<u>xylY</u>	CTGATTACGGCCAAGAAGGTCGCTGAAGAACGACTACATCGGCAAGTTCTGGATGATACCAACGCTGAGGTTGCTGAGTGGGAGACTGTTGCTGATGCAAGCC											
<u>benB</u>	AAAATCTTGAGCAAGTATGTTGCTTAAGAATGACTATATAATCAAGTCTGGATTTGAGATGGGCTTACCCGTTTATCTGATCGCTCAAGGTGAACCTTATCCGATGCGACATCG											
2170	2180	2190	2200	2210	2220	2230	2240					
XylY	LeuIleThrAlaLysValValLeuLysAsnAspTyrIleArgGlnValIleAspValTyrHisVal	*	*	*	*	*	*					
BenB	LysIleLeuSerLysTyrValMetLeuLysAsnAspTyrIleAsnGlnValIleAspIleTyrHisIle	*	*	*	*	*	*					
BenC												
	2330	2340	2350	2360	2370	2380	2390	2400	2410	2420		
<u>xylZ</u>	-----TGCCCCATGACACACAAAGGTTGCGACTGACTTCGAAGACGGCGTCACTCGTTCATCGATGCTAACTGCGAGACTGTTGCTGATGCAAGCC											
<u>benC</u>	TTATTTGAATAGGATTCTGCGCATGTCACCATGAGTAGCTACGCTAACGTTGAAGATGGGCTTACCCGTTTATCTGATCGCTCAAGGTGAACCTTATCCGATGCGACATCG											
2290	2300	2310	2320	2330	2340	2350	2360	2370	2380	2390	2400	
XylZ	MetThrHisLysValAlaThrAspPheGluAspGlyValThrArgPheIleAspAlaAsnThrGlyGluThrValAlaAspAlaTyrArg	*	*	*	*	*	*	*	*	*	*	*
BenC	LeuTyrLeuAsnArgIleProAlaMetSerAsnHisGlnValAlaLeuGlnPheGluAspGlyValThrArgPheIleCysIleAlaGlnGlyGluThrLeuSerAspAlaAlaTyrArg	*	*	*	*	*	*	*	*	*	*	*

FIG. 1—Continued.

2430 2440 2450 2460 2470 2480 2490 2500 2510 2520 2530 2540
xylZ CAAGGCATCAATTACCCCTGGACTGCCGAGACGGTGCATGCCGCCCTGCAAATGCTTCGCTGAGAGCGCCGCTACAGCCTCGCGGAGGAG--TATATCAGGATGCACTTAGCGAA
benD CAGCAAATCAATTACCAATGGACTGCCGTGAAGGCCGAGTGTGGTACCTGCCGTCTTTTGTAATCGGCAACTATGACATGCCGAAAGACAATTACATGAAAGATGCACTACCCCCA
 2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520
 XylZ GluGlyIleAsnLeuProLeuAspCysArgAspGlyAlaCysGlyAlaCysLysCysPheAlaGluSerGlyArgTyrSerLeuGluGluGlu---TyrIleGluAspAlaLeuSerGlu
 *
 BenC GlnGlnIleAsnileProMetAspCysArgGluGlyGlucysGlyThrCysArgAlaPheCysGluSerGlyAsnTyrAspMetProGluAspAsnTyrIleGluAspAlaLeuThrPro
 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640 2650 2660
xylZ GCCGAACGCCGAGCAGGGTACGTGCTGACCTGCCAGATGCCGCCAGCAGCGACTCGCTGATTGCCGTTCCGGCCATCGGAGCTGCAAGGCCAGCAGGCCGCTATCAGGCACCG
benC GAAGAACGCCGAGCAGGGTACGTGCTGACCTGCCAGATGCCGCCAGCAGCGACTCGCTGATTGCCGTTCCGGCCATCGGAGCTGCAAGGCCAGCAGGCCGCTATCAGGCACCG
 2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
 XylZ AlaGluAlaGluGlnGlyTyrValLeuThrCysGlnMetArgAlaGluSerAspCysValIleArgValProAlaAlaSerAspValCysLysThrGlnGlnAlaGlyTyrGlnAlaAla
 *
 BenC GluGluAlaGlnGlnGlyTyrValLeuAlaCysGlnCysArgProThrSerAspAlaValPheGlnIleGlnAlaSerSerGluValCysLysThrLysIleHisHisPheGluGlyThr
 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760 2770
xylZ ATCAGAACGTCGCTAGCTGCCGAGACGACCATGCCGTGCTATAAAAGGCCATGCTGAA---CCAGTGGCTTCTGCCAGGCACTGCAAGTGCAGGCACTGCAAGTGCAGGCAAC
benC ATGGCCGGGGTGTAAAATCTCGATTGACCCATCACCTTGATATTGAGCTGATGACGGTCAAGGGATATTGATTTCTGGCAGGGAGTATGCAACGTGACGCTGAGGCAACGGCACC
 2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760
 XylZ IleSerAsnValArgGinLeuSerGluSerThrIleAlaLeuSerIleLysSerAlaSerLeuAsn---GlnLeuAlaPheLeuProGlyGlnTyrValAsnLeuGlnValProGlySer
 *
 BenC LeuAlaArgValGluAsnLeuSerAspSerThrIleThrPheAspIleGlnLeuAspAspGlyGlnProAspIleHisPheLeuAlaGlyGlnTyrValAsnValThrLeuProGlyThr
 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880 2890
xylZ GACCAACCCGGCCATTCTTCAGTCGCTGCAAAGGATGGCAGGGTCACTCTCTGATCCGCAAGCTGCCGGCGGGCTGATGAGGCACTTCTGACACGCTCGCCAAGGTCGG
benC ACGGAAACACGCTGTATTGTTAGCTCACACCGCAATCGCTAACCGGGTTCTGTTGCTGAAATGCCCAGGTTAAATGAGGCAATATTAAAGTGTGAGGCAACGGCACCAGCAGC
 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880
 XylZ AspGlnThrArgAlaTyrSerPheSerSerLeuGlnLysAspGlyGluValSerPheLeuIleArgLysLeuProGlyGlyLeuMetSerSerPheLeuThrSerLeuAlaLysValGly
 *
 BenC ThrGluThrArgSerTyrSerPheSerGlnProGlyAsnArgLeuThrGlyPheValValArgAsnValProGlnGlyLysMetSerGluTyrLeuSerValGlnAlaLysAlaLys
 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000 3010
xylZ GATAGCGTCAGTCTGGCTGGACCGCTGGCGCGTTCTATGCGCAGATCAAGCGCCGCTGCTGTTGCGCCGCTACCGGCCATCGGCGATCACCCTGAGGAGAAGATC
benC GACAAAATGAGCTTTACTGGACCATTTGGTAGTTTATCTGGCTGATGTCAGCGCTCTGCTCATGCTGGCTGGCGTACGGGAATCGCACCGTTTATCGATGTTGCAAGTACT
 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
 XylZ AspSerValSerLeuAlaGlyProLeuGlyAlaPheTyrLeuArgGluIleLysArgProLeuLeuLeuAlaGlyGlyThrGlyLeuAlaProPheThrAlaMetLeuGluLysIle
 *
 BenC AspLysMetSerPheThrGlyProPheGlySerPheTyrLeuArgAspValLeuMetLeuAlaGlyGlyThrGlyIleAlaProPheLeuSerMetLeuGlnValLeu
 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120 3130
xylZ GCCGAGCAGGGCGCGAGCACCCGCTCCATCTGATCTACGGCGTACCCCATGACCAAGCTGGTGAATGAGCAAGCTAGAGGCACTTCCCGCGCGCATCCAACTTCAGCTACAGC
benC GAGCAAAAAGGCAAGTGAAGCATCCAGTACGACTGGTTGGCGTAACCCAAGATGTGATCTGGTGGCGCTGAACAACTCGACTTCAGCAGAAACTACCATGGTTGAATATCGT
 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120
 XylZ AlaGluGlnGlyGlyGluHisLeuIleTyrGlyValThrHisAspHisAspLeuValGluMetAspLysLeuGluAlaPheAlaAlaArgIleProAsnPheSerTyrSer
 *
 BenC GluGlnLysGlySerGluHisProValArgLeuValPheGlyValThrGlnAspCysAspLeuValAlaLeuGluGlnIleLeuAspAlaLeuGlnGlnLysLeuProTrpPheGluTyrArg
 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240 3250
xylZ GCCTCGCTGCCAGCCAGACGCCCTATCCGCAAAGGCTATGTGACCCAGTACATCGAGGCCAACAGCTAACGCCGGTGAGGTTAGATCTACCTTGGCTCCGCCACCGATG
benC ACCGGGGTGGCACATCGAGAAAGTCAACATGAACGTAAGGTTACGTGACGGGTATATCGAAATATGACTGGCTAAATGGCGTGAAGTTGATGTTGATCTGGCGACCGGTTCTATG
 3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240
 XylZ AlaCysValAlaSerProAspSerAlaTyrProGlnLysGlyTyrValLeuIleGluGlyIleArgLysLeuProAlaAspPheTyrTyrGluLysPheAlaAlaSerAla
 *
 BenC ThrValValAlaHisAlaGluSerGlnNisGluArgLysGlyTyrValThrGlyHisIleGluTyrAspTrpLeuAsnGlyGlyGluValAspValTyrLeuCysGlyProValProMet
 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360 3370
xylZ GTGGAGGGCGCTAGTCAGTACATCGGGCACAGGGATCCAGCCGCCAATTCTATTATGAGAAGTTCGCCGCCAGCGCCTAGAGGCTCTTGGTCCGCTTACTAGGTGAGGCGGGCT
benC GTGGAGGGCGCTGGAGCTGGCTGGATACGCAAGGTTACCAACCGGCAGACTTTTATTGAAAGGAAATTCTCTGCCAACTAA---
 3250 3260 3270 3280 3290 3300 3310 3320
 XylZ ValGluAlaValSerGlnIleArgAlaGlnGlyIleGlnProAlaAspPheTyrTyrGluLysPheAlaAlaSerAla
 *
 BenC ValGluAlaValArgSerTrpLeuAspThrGlnGlyIleGlnProAlaAsnPheLeuPheGluLysPheSerAlaAsn
 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480 3490
xylL GTTTTATTGGCCAGTGGCCAGGTTCTCTGCCAACCTTCCAAATGCTCCGGCTACACAACCGAGGTGGTCTGACAAACGTTCCAGGGCAGGTTGCCGTTACCCGGC
benD CTCAAAACGGAGGCGATATGAAATTCGACACAAACGTTGATGAGATCAAGGCTTCTGATCTGAGATCAAGGCTTCTGATCTGAGATCAAGGCTTCTGATCTGAGATCAAGGCTTCTG
 3330 3340 3350 3360 3370 3380 3390
 XylL MetAsnLysArgPheGlnGlyLysValAlaValIleValThrGly
 BerD
 NetAsnSerThrGlnArgPheGluHisLysValValIleValThrGly
 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600 3610
xylL GCGCCGCCAGGGCATCGGTGCCGCCGAGGGATGCCGCCAGGGCTGCCGCCGAGGGCTGCCGCCGAGGGCTACAGGCTAATACATGAA---GCTGGCCGACGAACTGGTCCGAGCTC
benD GCAGCTCAAGGATTGGTCTGGTGTGACTACGGATTGCCAACAGGGAGGGTCTGATATTGGCCGACGGCTTCTGATCTGAGATCAAGGCTTCTGATCTGAGATCAAGGCTTCTG
 3400 3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
 XylL AlaAlaGlnGlyIleGlyArgArgValAlaGluArgMetAlaAlaGluGlyGlyArgLeuLeuValAspArgSerGluLeuIleHisGlu---LeuAlaAspGluLeuValGlyVal
 BerD AlaAlaGlnGlyIleGlyArgGlyValAlaLeuArgIleAlaGlnGluGlyCysLeuIleLeuAlaAspAspSerAspIleGlnIleValAlaLeuIleValAlaLeuIle

FIG. 1—Continued.

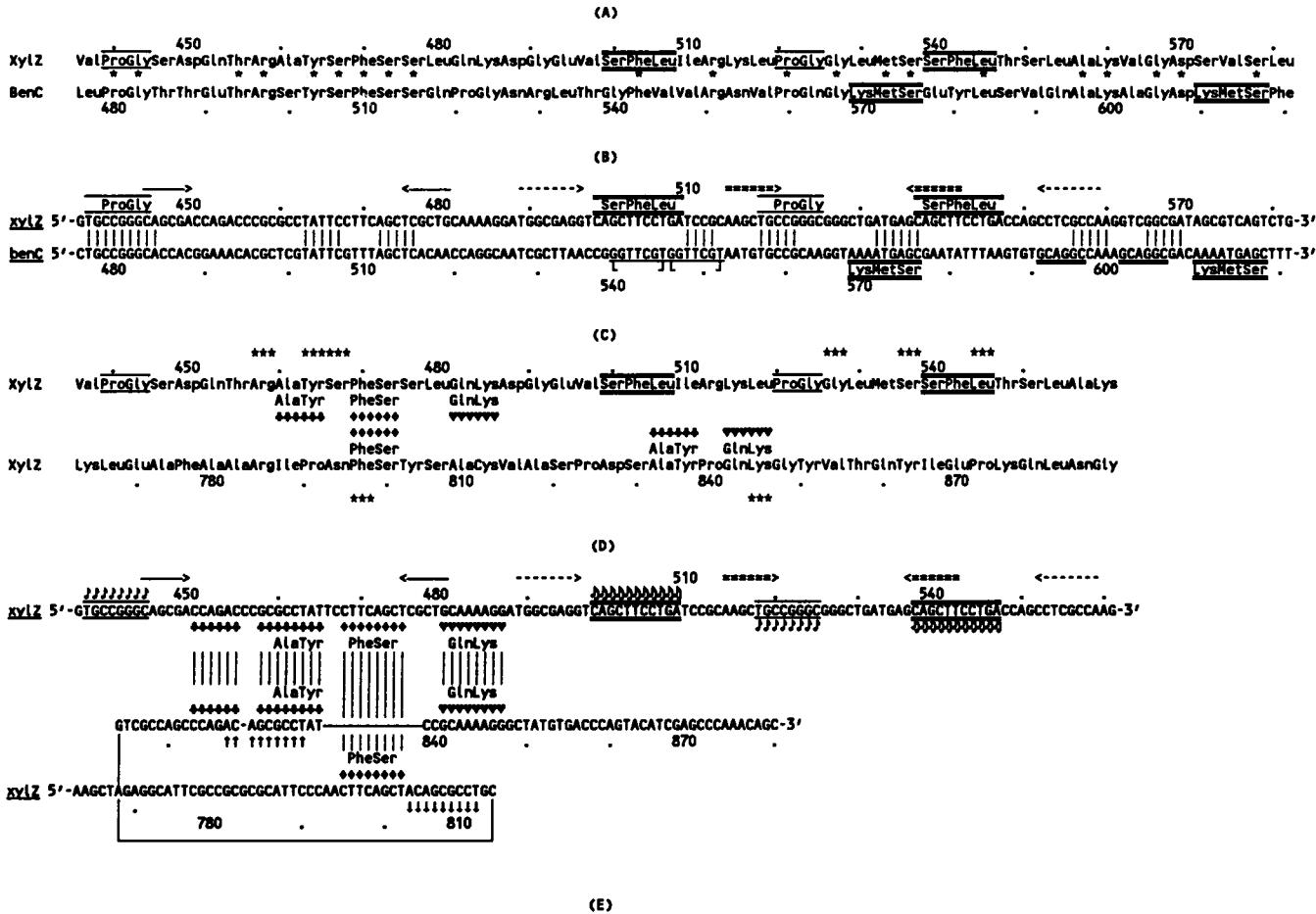


FIG. 2. Inferences concerning common ancestry of DNA sequence repetitions acquired during evolution of upstream (positions 436 to 561) and downstream (positions 763 to 888) regions of *xylZ*. Numerals correspond to positions within the nucleotide sequence of the genes. (A) Amino acid sequence repetitions within the upstream region of *XylZ* and within the homologous region of *BenC*. Asterisks mark amino acids conserved in the two gene products. Repeated peptides within each divergent amino acid sequence are marked above and below by single or double lines. (B) DNA sequence repetitions acquired within regions of *xylZ* and *benC* and corresponding to the amino acid sequence comparisons shown in panel A. Vertical lines connect regions in which four or more contiguous bases in the aligned *xylZ* and *benC* DNA sequences are identical. Horizontal single or double lines indicate direct DNA sequence repetitions. Inverted DNA sequence repetitions within *xylZ* are distinctively marked with arrows. (C) Dipeptides found in the upstream and downstream regions of *XylZ* are marked with clubs (AlaTyr), diamonds (PheSer), and hearts (GlnLys). Stars above and below the amino acid sequences indicate amino acids that have been conserved during divergence of *XylZ* and *BenC* from ancestors they share with chloroplast ferredoxin reductase (22). (D) Introduction of a loop into the downstream *xylZ* sequence suggests how DNA encoding peptides conserved between the upstream and downstream sequences could be traced to a slippage structure acquired during divergence of the gene. Clubs, diamonds, and hearts indicate DNA corresponding to peptides depicted in panel C. Single or double musical notes mark DNA sequences corresponding to peptides repeated in the upstream region as shown in panel B, and vertical arrows indicate a DNA sequence repetition acquired in the downstream region. Vertical lines connect DNA sequence identities clustered in the aligned regions of the upstream and downstream sequences. (E) Frequency of usage within *xylZ* of codons for peptides repeated within the upstream and downstream regions.

The complex nature of DNA slippage structures and their potential ability to interact makes them difficult to elucidate. Procedures that may reveal such structures are illustrated by analysis of repetitions acquired in *xylZ* during its divergence from an ancestral gene it shared with *benC*. The *xylZ* sequence repetitions presented in Fig. 2 and 3 encode the C-terminal region of *XylZ* and were acquired during divergence of the protein from predecessors shared with other oxidoreductases including ferredoxin reductase and, more recently, *BenC*.

DNA sequences presented as slippage structures in Fig. 2

and 3 were selected after screening the entire *xylXYZ* region for DNA sequence repetitions exceeding 8 bp and falling within 120 bp of each other. Only two DNA segments, the upstream and downstream sequences in Fig. 2 and 3, met this stringent criterion for evidence of localized strand slippage. This inference was strengthened by discovery of three nonoverlapping pairs of inverted repetitions of 6 bp or longer within the upstream region. Further analysis revealed three different 8-bp sequences in the upstream region that were repeated, albeit in different order, in the downstream region. Potential hybridization patterns between comple-

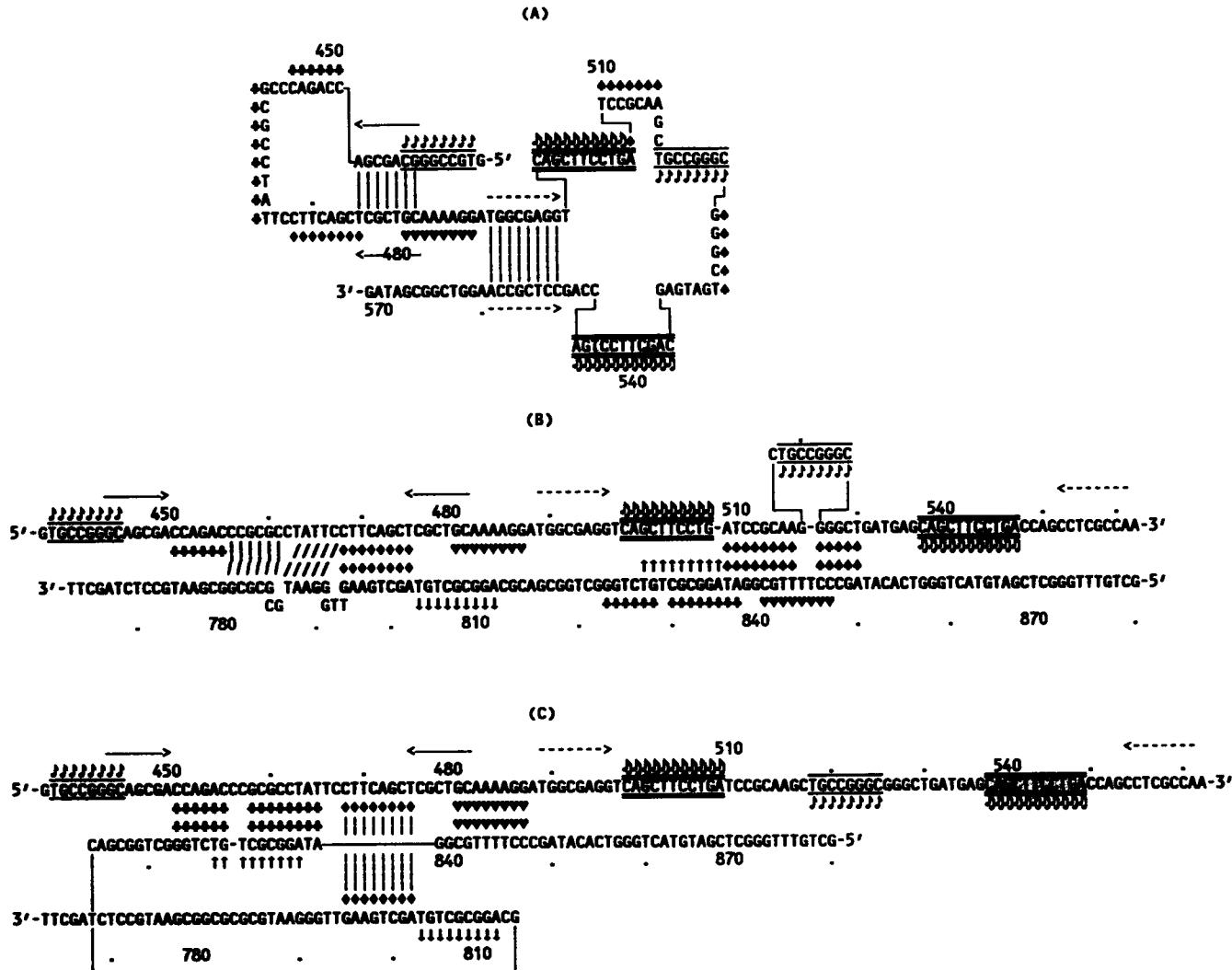


FIG. 3. Slippage structures suggested by repeated DNA sequences in the upstream and downstream regions of *xylZ*. (A) The 5' (coding) sequence of the upstream region (Fig. 2) is folded so that vertical lines illustrate hybridization that might occur between inverted repetitions. As in Fig. 2, single or double musical notes indicate direct DNA sequence repetitions that might allow hybridization between the coding and noncoding strands of the upstream region after slippage. Largely single stranded within the coding strand slippage structure are sequences (clubs, diamonds, and hearts) that possess the potential ability to hybridize with the noncoding strand of the downstream region of *xylZ* (Fig. 2D and panel C, below). Spades indicate DNA which, pinched into single strandedness in this representation of the upstream region, has the potential ability to initiate formation of a slippage structure by hybridizing with DNA in the downstream region as shown in panel B. (B) One possible DNA slippage structure in which hybridization between the coding strand of the upstream region and the noncoding strand of the downstream region might predominate. Horizontal arrows indicate regions contributing to the intrastrand loops depicted in panel A. Vertical arrows mark regions of direct sequence repetition, separated by 24 bases, that could allow hybridization between coding and noncoding strands of the downstream region. This interaction might contribute to formation of the alternative slippage structure shown in panel C. (C) Slippage structure that could account for conservation of DNA sequence repetitions within *xylZ* as depicted in Fig. 2D.

mentary components of repeated DNA sequences are presented in Fig. 3.

A portion of the amino acid sequence of XylZ is compared with the corresponding sequence of BenC in Fig. 2A. The comparison illustrates the similarities that run throughout the aligned sequences (Fig. 1) and demonstrates internal repetitions, expressed at the level of peptide sequence, that were acquired as XylZ and BenC diverged. Figure 2B shows that the acquired repetitions observed at the level of peptide in Fig. 2A are expressed more extensively at the level of direct DNA sequence repetitions which are as long as 11 bp (in the *xylZ* sequences encoding SerPheLeu, Fig. 2B). In

addition, the *xylZ* DNA contains inverted repetitions lying in regions divergent from *benC* (Fig. 2B).

The XylZ sequence contains three dipeptides which are repeated in a different order in a downstream portion of the same gene product (Fig. 2C). As shown in Fig. 2D, introduction of a loop into the 5' strand encoding the downstream set of dipeptides allows alignment of its DNA with a nearly identical sequence encoding the upstream set of peptides. Also shown in Fig. 2D are direct and inverted DNA sequence repetitions that have the potential ability to hybridize and thus to contribute to slippage structures. The frequency of codon usage for the repeated dipeptides, shown in Fig.

2E, indicates that the probability that this particular DNA sequence would be used to encode the peptides is 0.026. This value is a substantial overestimate because it overlooks the fact that DNA encoding the dipeptides represents repetitions extending 8 rather than 6 bp (Fig. 2D). Furthermore, the 8-bp repetition corresponding to the peptide AlaTyr is flanked by a perfect 6-bp repetition between the upstream and downstream sequences (Fig. 2D).

Clusters of DNA sequence identity shared between the upstream and downstream regions of *xyl/Z* (Fig. 2D) are more extensive than those shared by the upstream region of *xyl/Z* and its unmistakable *benC* homolog (Fig. 2B): the former comparison contains three clusters in which sequence identities exceed seven contiguous base pairs, and only one such cluster is found in the latter comparison. This evidence points to the conclusion that the upstream and downstream regions of *xyl/Z* share common ancestry, and this common ancestry was established by genetic exchange between the regions subsequent to their divergence from the ancestor shared with *benC*. The genetic exchange must have been based on hybridization between complementary sequences in the upstream and downstream regions of *xyl/Z*; examination of the DNA sequences in these regions (Fig. 3) suggests how such hybridization might be formed.

In Fig. 3A, the 5' (coding) strand from the upstream region of *xyl/Z* is depicted in a form it would possess if hybridization between inverted repetitions were allowed to take place. The 3' (noncoding) strand is not shown in this depiction, but it should be recognized that it could enter into a slippage structure through hybridization between complementary components of direct DNA sequence repetitions. The coding portions of these repetitions, represented by single or double musical notes in Fig. 3A, are largely single stranded and therefore accessible for interaction with their misaligned noncoding counterparts in the upstream region. Also largely single stranded in the depicted structure of the upstream coding region are DNA sequences (symbolized by clubs, diamonds, and hearts in Fig. 3A) that are free to enter into hybridization with noncoding counterparts in the downstream region (Fig. 3C).

The remaining portion of the upstream coding strand is represented largely by the sequence marked by spades in Fig. 3A. As shown in Fig. 3B, this region could enter into hybridization with a portion of the noncoding downstream strand to form an alternative slippage structure. This structure would pinch into single strandedness the coding component of the upstream direct repetition that is represented by single musical notes (Fig. 3B). Also forced into single strandedness by the slippage structure suggested in Fig. 3B would be noncoding DNA (symbolized by hearts in the downstream region) that would have potential ability to hybridize with coding DNA (symbolized by hearts in the upstream region) and thus contribute to formation of the slippage structure shown in Fig. 3C.

Existence of the potentially interactive slippage structures shown in Fig. 2 and 3 is proposed on the basis of patterns of sequence identity that appear to defy coincidence. Regions of sequence identity in misaligned DNA strands extend from 6 to 8 bp and fit into three-dimensional jigsaw puzzles in which very few pieces appear to be missing (Fig. 2 and 3). The repeated DNA sequences, acquired during divergence of *xyl/Z* from the ancestor it shared with *benC* (Fig. 2), have been maintained even against selective pressure for usage of preferred codons. It therefore seems possible that mismatch repair between slipped DNA strands contributes to maintenance of the DNA sequence repetitions. This possibility can

be explored by examination of the stability of mutations that disrupt potentially interactive DNA sequences and also cause substitution of essential amino acid residues in proteins. The contribution of slippage structures to repair of such mutations could be assessed by examination of the reversion frequency of strains in which they are carried.

Site-directed mutagenesis (15) can be used to prepare bacterial strains carrying mutations that alter amino acid residues essential for *xyl/Z* function. Targets for such mutagenesis emerge from identification of amino acid residues conserved within the broad oxidoreductase family encompassing chloroplast ferredoxin reductase (22). Targets thus identified are marked with stars in the slippage structure suggested in Fig. 2. The specific contribution made by the targeted residues can be inferred because the crystal structure of ferredoxin reductase has been determined (14). The likelihood that these residues were conserved because they make an important contribution to enzyme function can be tested by their genetic modification through site-directed mutagenesis. If the modifications result in an inactive enzyme, the possibility that slippage structures contribute to DNA sequence maintenance can be explored by examining frequencies of reversion of the mutated DNA.

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