Molecular analysis of the DNA *gyrB* gene from *Myxococcus xanthus*

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DNA gyrase, an essential type II topoisomerase, mediates negative supercoiling of the bacterial chromosome, thereby affecting the processes of DNA replication, transcription, recombination and repair. The *gyrB* gene from the Gram-negative soil bacterium *Myxococcus xanthus* was sequenced. The sequence predicts a protein of 815 amino acid residues displaying significant homology to all known GyrB proteins. A 6-His-GyrB fusion protein was overexpressed in *Escherichia coli* and purified to near homogeneity using affinity chromatography on Ni-nitrilotriacetic acid-agarose and novobiocin-Sepharose columns. The fusion protein bound novobiocin and cross-reacted with anti-*E. coli* GyrB antibodies, indicating structural and functional similarities to the *E. coli* DNA GyrB. The gene was mapped to the region of the origin of replication (*oriC*) of *M. xanthus*.

Keywords: *Myxococcus xanthus*, DNA gyrase B, topoisomerase

INTRODUCTION

DNA topoisomerases play a crucial role in cellular processes that depend on the superhelical density of DNA, such as DNA replication, transcription, recombination and repair (see Orr *et al.*, 1984; Wang, 1996). Although all known topoisomerases can relax negatively supercoiled DNA, gyrase is the only type II topoisomerase that catalyses the ATP-dependent conversion of relaxed duplex DNA to its negatively superhelical form. The functional DNA gyrase holoenzyme is a tetrameric protein (A_2B_2) composed of two A and two B subunits encoded by the *gyrA* and *gyrB* genes, respectively. The 90–100 kDa GyrA subunit promotes the transient breakage and reunion of DNA, whereas the 85–95 kDa GyrB catalyses the hydrolysis of ATP, providing the energy required for the supercoiling reaction (Wang, 1996). The enzyme is the target for two groups of antimicrobial drugs, the 4-quinolones (Crumplin, 1990) and the coumarins (Gellert *et al.*, 1976), which inhibit the activities of GyrA and GyrB, respectively.

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Abbreviation: NTA, nitrilotriacetic acid.

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The sequences of genes encoding either one or both subunits (*gyrA* and *gyrB*) of DNA gyrase from several bacteria, including *Escherichia coli* (Adachi *et al.*, 1987; Swanberg & Wang, 1987), *Pseudomonas putida* (Parales & Harwood, 1990), *Bacillus subtilis* (Moriya *et al.*, 1985), *Haloferax* sp. (Holmes & Dyll-Smith, 1991), *Staphylococcus aureus* (Margerrison *et al.*, 1992) and *Haemophilus influenzae* (Fleischmann *et al.*, 1995), have been determined and analysed. The studies indicate that, in prokaryotes, both genes are structurally conserved reflecting their functional homology. Furthermore, in many bacteria analysed so far, the organization of genes around the *gyrB* gene is highly conserved and found in the order *rnpA-rpmH-dnaA-dnaN-recF-gyrB*, with the exception of a few, such as *Borrelia burgdorferi* (Old *et al.*, 1993), *Mycoplasma capricolum* (Miyata *et al.*, 1993) and *H. influenzae* (Fleischmann *et al.*, 1995). The origin of chromosome replication (*oriC*) frequently resides in the *dnaA* region, as observed for *B. subtilis* (Ogasawara *et al.*, 1985), *Salmonella typhimurium* (O'Brien, 1990) and possibly *P. putida* (Fujita *et al.*, 1989). In *E. coli*, *oriC* is located approximately 40 kb away from the *dnaA* gene, presumably due to an inversion event (Yoshikawa & Ogasawara, 1991). Several new bacterial type II topoisomerases have been identified (Wang, 1996). The *E. coli* *parC* and *parE* (topoisomerase IV) genes are homologous to the *gyrA* and *gyrB* genes, respectively, and are involved in chromosomal partition. The ParE,

however, is smaller than the GyrB, lacking the latter C-terminal domain.

Myxococcus xanthus is a Gram-negative, gliding, soil bacterium that feeds on proteins and peptides. It resembles the eukaryotic cellular slime moulds in its ability to form multicellular fruiting bodies during development. The formation of a fruiting body is a process by which tens of thousands of cells move, upon starvation, towards an aggregation centre to form a multicellular structure (Reichenbach, 1993). In this structure, individual rod-shaped cells differentiate into round, dormant, environmentally resistant myxospores. Genetic and biochemical evidence indicate that essential intercellular signalling occurs at multiple stages during the developmental process and that cell to cell interactions are required for proper transcriptional regulation of developmental genes.

The physical map of the 9454 kb *M. xanthus* chromosome was constructed using a yeast artificial chromosome (YAC) library and restriction patterns (Chen *et al.*, 1991; He *et al.*, 1994). The *oriC* of *M. xanthus* was identified on a 330 kb fragment (Komano *et al.*, 1985) within the chromosome map (Chen *et al.*, 1991; He *et al.*, 1994; Kuspa *et al.*, 1989), though the detailed organization of the genes around *oriC* is as yet unknown.

During the analysis of gene clusters in *M. xanthus*, we discovered a novel sequence homologous to bacterial *gyrB* genes. Here we provide evidence for the structural and functional homology of its product to other GyrB proteins.

METHODS

Bacterial strains and plasmids. The *M. xanthus* strain used in this study is ER-15, a mutant producing red pigment (Varon *et al.*, 1992). *E. coli* TG1, DH10 β and XL-1 Blue MR were used for cloning and for DNA manipulation. *E. coli* M15(pREP4) (Qiagen) was used for overexpression of the 6-His-GyrB fusion protein. The *E. coli gyrB* (temperature-sensitive) mutant LE316 (Orr *et al.*, 1979) was utilized for the complementation assay of the *M. xanthus* GyrB *in vivo*. A conjugative tagged Tn1000 transposition system was applied in conjunction with the *E. coli* strains MH1578 and MH1599 for the sequencing as described previously (Sedgwick & Morgan, 1994). The vectors pUC18, pUC19 (Norrander *et al.*, 1983) and SuperCos 1 (Stratagene) were used for cloning and sequencing and the vector pQE9 (Qiagen) was used for the expression of the 6-His-GyrB fusion protein.

Media and growth conditions. *E. coli* was grown in Luria broth (LB), on LB agar or in minimal media, at the required temperature, with the appropriate antibiotics as described previously by Sambrook *et al.* (1989). *M. xanthus* was grown at 32 °C in 1 CT medium (Tolchinsky *et al.*, 1992; Varon *et al.*, 1992), or on the same medium solidified by 1.5% Bacto agar (Difco).

General DNA procedures. Standard genetic techniques, Southern blot analysis, colony hybridization, plasmid preparations and *in vitro* DNA manipulations were as described previously by Sambrook *et al.* (1989). The isolation of total DNA from *M. xanthus* has been described elsewhere

(Avery & Kaiser, 1983). Cosmid DNA and plasmid DNA templates for sequencing reactions were purified using Qiagen columns. Conjugative transposition of Tn1000 for DNA sequencing was performed as described previously by Sedgwick & Morgan (1994).

DNA sequencing and analysis. Automated DNA sequencing was carried out on double-stranded DNA templates, using the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977), with an Applied Biosystems model 373A sequencer. The cosmid clone pPYCA111 (a recombinant cosmid containing the *gyrB* region) was subcloned into the vectors pUC18 and pUC19. A 3381 bp fragment derived from the cosmid pPYCA111 and the plasmid pPY05 was sequenced using the conjugative transposon mutagenesis walking system Tn1000 (Sedgwick & Morgan, 1994). Occasional sequence gaps were filled by direct sequencing using synthetic oligonucleotide primers. Each strand of the insert was sequenced at least three times in both directions. The assembly and analysis of the primary DNA sequence data were completed using the MacVector 3.5 (International Biotechnologies) and Sequence Navigator (Applied Biosystems) software. The search for DNA and protein homologies in the databases was achieved through the BLAST (Altschul *et al.*, 1990) and FASTA (Pearson, 1990) programs.

General protein procedures. The procedure for protein extraction from *E. coli* and *M. xanthus* was similar to that described previously (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989), or to the manufacturer's instructions (Qiagen; for the purification of the 6-His-GyrB fusion protein). Proteins were separated using SDS-PAGE and stained with Coomassie brilliant blue (Laemmli, 1970). Western blot analysis was performed as described elsewhere (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

Overexpression of GyrB. To construct a recombinant plasmid overexpressing a 6-His-GyrB fusion protein, a 2470 bp fragment containing the entire *gyrB* gene was amplified using a proof-reading DNA polymerase (ELONGASE Enzyme Mix; Gibco-BRL) from the cosmid pPYCA171 as instructed by the manufacturer. The reaction mixture was incubated at 94 °C for 5 min, followed by 25 cycles of: (1) denaturation at 94 °C for 1 min, (2) annealing at 50 °C for 30 s and (3) extension at 68 °C for 3 min. The reaction was finally incubated for 7 min at 68 °C. The forward primer (P1) was 5'CGGGATCCATG-GAAAAGACCCCGCTACC3', in which a *Bam*HI site (underlined) was incorporated so as to form an in-frame fusion immediately upstream of the first ATG initiation codon (in bold). The reverse primer (P2) was 5'GGGAAGCTTCG-CGTCTTTCCGTGGGACGG3' with a *Hind*III site (underlined) incorporated. The PCR product was purified with the Qiagen PCR purification kit, digested with *Bam*HI and *Hind*III, recovered from a 0.8% agarose gel after electrophoresis and cloned into the vector pQE9 (Qiagen). The resulting plasmid, pPYGB, carries the *gyrB* gene fused, in-frame, to the 6-His sequence at its 5' end and expressed under the control of the promoter/operator element, consisting of the *E. coli* phage T5 promoter and two *lac* operator sequences. pPYGB was transformed into *E. coli* M15(pREP4) (Qiagen) and single colonies were screened for the production of the GyrB fusion protein as instructed by the manufacturer (Qiagen) and analysed using 10% SDS-PAGE (Laemmli, 1970).

Isolation of the GyrB fusion protein. Highly purified 6-His-GyrB fusion protein (large- and small-scale preparations) was obtained by affinity chromatography on an Ni-nitrilotriacetic acid (NTA) resin column, following the manu-

Fig. 1. Physical map of the inserts of pPYCA171 (36 kb) and the *gyrB* region of pPYCA111. (a) Restriction map of cosmids pPYCA171 and pPYCA111; the sequenced 3.4 kb fragment is indicated by a black bar on pPYCA111. (b) The 2.6 kb *SalI* fragment cloned into the plasmid pPY05, and the primer P5F2 (5'-CCATCCACGTGGATGACG-ACC-3'). (c) Primers P1 and P2, used for the amplification of the *gyrB* gene from cosmid pPYCA171 and cloning in to pPQE9. Restriction enzyme sites: S, *SalI*; X, *XhoI*; Bm, *BamHI*.

facturer's instructions (Qiagen), and affinity chromatography on novobiocin-Sepharose as described previously (Orr & Staudenbauer, 1982; Staudenbauer & Orr, 1981).

RESULTS

Cloning the *gyrB* gene from *M. xanthus*

In the course of constructing a cosmid library of *M. xanthus*, we analysed the recombinant cosmid pPYCA111 (Fig. 1a) and subcloned its insert into the vectors pUC18 and pUC19. Partial sequence analysis of a 2.6 kb *SalI* fragment subcloned into pUC18 (pPY05; Fig. 1b) showed that it contained a sequence homologous to various *gyrB* genes. The 5' end of the sequence was identified in the coding region of the gene and, therefore, was presumed to lack its start codons. A high stringency Southern hybridization using the 2.6 kb *SalI* fragment to probe *SalI*-digested chromosomal DNA indicated the existence of only one identical fragment in the chromosome of *M. xanthus* ER-15 (data not shown). The entire *gyrB* gene was consequently obtained from a cosmid library of *M. xanthus* ER-15, probed with the 2.6 kb *SalI* fragment of pPY05. The positive recombinant pPYCA171 which contained the 2.6 kb *SalI* fragment was further characterized by restriction enzyme digests (Fig. 1a).

Sequence analysis of the *M. xanthus gyrB* gene

The sequence of the 5' end of the putative *gyrB* gene was determined by using the cosmid pPYCA111 as a template and the synthetic oligonucleotide primer P5F2 (Fig. 1b). The sequence consists of 3381 bp with a G + C content of 66.7 mol%, in line with the composition of myxobacterial DNA (Shimkets, 1993). The frequency of G + C at the third position of each codon in the putative ORF (see below) was found to be 89.1 mol%, a typical value for an ORF in organisms with a high percentage of G + C. The *M. xanthus* ORF could potentially encode a protein of 815 amino acids with a molecular mass of 89.63 kDa. A putative ribosome-binding site (AAGGA) was found 9 bp upstream of the initiation (ATG) codon, and a putative terminator was found at the 3' end.

Consensus sequences of bacterial promoters at -10 and -35 (such as TATAAT and TTGACA, respectively, for *E. coli*) could not be identified.

The deduced amino acid sequence of the *M. xanthus* ORF displays strong homology to all GyrB proteins. The highest score was found with the products of *S. typhimurium gyrB* (49.8% identity, 58.1% similarity) and *E. coli gyrB* (49.7% identity, 58.1% similarity) (Fig. 2). The identity to the smaller *E. coli* protein ParE was only 39.7% (48.8% similarity). Based on the amino acid sequence homology and the predicted protein size, we concluded that this *M. xanthus* ORF is the *gyrB* gene.

Mapping the *gyrB* gene on the *M. xanthus* chromosome

A 2.1 kb fragment, containing most of the *gyrB* gene, was amplified from the plasmid pPY05 (Fig. 1b) by PCR. This fragment was used as a probe to screen an *M. xanthus* YAC library (kindly performed by H. Hendrix & L. J. Shimkets, University of Georgia, Athens, GA, USA) (Chen *et al.*, 1991; He *et al.*, 1994; Kuspa *et al.*, 1989). Two YAC plasmids, nos 1730 and 931 (He *et al.*, 1994), both located within the *oriC* region of *M. xanthus*, hybridized to the *gyrB* probe. The finding that the *gyrB* gene of *M. xanthus* is located near the chromosomal origin of replication is compatible with similar findings in other bacteria. It is therefore feasible that the *M. xanthus gyrB* gene is part of a gene cluster containing the genes *rnpA-rpmH-dnaA-dnaN-recF-gyrB* that is highly conserved in many bacteria and is frequently located close to *oriC* (Calcutt, 1994; Ladefoged & Christiansen, 1994; Ogasawara *et al.*, 1990).

Overexpression of the GyrB protein

To construct a recombinant plasmid overexpressing a 6-His-GyrB fusion protein, a 2470 bp fragment containing the entire *gyrB* gene (Fig. 1) was amplified by PCR from the cosmid pPYCA171 using synthetic oligonucleotides (see Methods). The forward primer, P1 (Fig. 1c), contained a *BamHI* restriction site upstream of the first

<i>M. xanthus</i>	1	MEKTPATGS AVAPPPVEYGTDSITVLEGEAVRKRPGMYIGDTM-AYGLHLKL	51
<i>E. coli</i>	1	-----MSNSYDSSSIKVLGLGLAVRKRPGMYIGDTDDGTGLHHM	39
<i>N. gonorrhoeae</i>	1	-----MTEQKH EYVY GADSIQVLEGLEAVRKRPGMYIGDTQDGSGLHHM	43
<i>S. aureus</i>	1	-----MSQDNE-YGAGCIQVLEGLEAVRKRPGMYIGST-DSRGLHHL	40
<i>B. subtilis</i>	1	-----MEQQQNSYDENCTQVLEGLEAVRKRPGMYIGSTN-SKGLHHL	41
<i>M. xanthus</i>	52	VYEVVDNAIDELAGHCCTDIEVVIHVHVGSLSVQDNGRGIFVGEHPKFFPKD.T	103
<i>E. coli</i>	40	VFEVVDNAIDELAGHCCKEITVITHADNSVSVQDNGRGIFGTGIHPPEE-IGVSA	90
<i>N. gonorrhoeae</i>	44	VFEVVDNAIDELAGHCCKEITVITHADNSVSVQDNGRGIFGTGIHPKE-IGRSA	94
<i>S. aureus</i>	41	VYEVVDNSIDEALAGHCDAIEVVAHEDGSLSVQDNGRGIFVGTGHEQY-DRPA	91
<i>B. subtilis</i>	42	VFEVVDNSIDEALAGHCCTDINICTEKNISITVVDNGRGIFVGTGHEKM-IGRPA	92
<i>M. xanthus</i>	104	LEVVIITLHAGGSKFGNGAYKVS GGLHGVGVTVVNLSSEWFKVRVHNGI VYE	155
<i>E. coli</i>	91	AEVIMTVLHAGGKFDNSYKVS GGLHGVGVSVVNALSOKLELVIQREGKIHR	142
<i>N. gonorrhoeae</i>	95	AEVIMTVLHAGGKFDNSYKVS GGLHGVGVSVVNALSOKLELVIQREGKIHR	146
<i>S. aureus</i>	92	LEVIMTVLHAGGKFDNSYKVS GGLHGVGVTVVNALSSELEVEVKKHGGAVVT	143
<i>B. subtilis</i>	93	VFEVIMTVLHAGGKFDNSYKVS GGLHGVGVSVVNALSSTELDVTVHEDGKIHR	144
<i>M. xanthus</i>	156	QAYACGVFDTPP---REVGT TDKRGTHIAFKPDATVM-ELVEFNFTETLSQR	202
<i>E. coli</i>	143	QIYEHGVLPQAP---LAVTGTETKTGT MVRFWPSLETFTNVTEFEYEILAKR	190
<i>N. gonorrhoeae</i>	147	VRFVVRGETE---EPLKIVSYGSDKKGT TVRFLAGTETFGNI-BYSFDILAKR	193
<i>S. aureus</i>	144	HRFEVGEHPQVEEFERVRDLEPGEDTGT TIRFWPDDGIF-ETTFEDFKTLENR	194
<i>B. subtilis</i>	145	QTYKRGVFDVTD---LEITGTETDHTGT TTHEVVDPELSETTBYDYDILANS	192
<i>M. xanthus</i>	203	LRELAFNLNAGLHIVVRDERTNKEH--DFKFDGGTSSFVEYLNKSKOTLHKDP	252
<i>E. coli</i>	191	LRELSFLNSGVSIIRLRDRDKGKE--DHFHYEGGJKAFVEYLNKSKOTLHPNI	240
<i>N. gonorrhoeae</i>	194	IRELSFLNNGVDIELTDERDGGKHE--FALSGGVAGFVYVNRKKTPLEKEI	243
<i>S. aureus</i>	195	LRELAFNLNAGLHIVVRDERTNKEH--DFKFDGGTSSFVEYLNKSKOTLHKDP	244
<i>B. subtilis</i>	193	VRELAETIKGMNTITIEKREGQERKNEYHYEGGJKSYVEYLNKSKOTLHKDP	244
<i>M. xanthus</i>	253	ISFSTEREGVMILDIAMOWNDGYDERITYTFANNIN THEGGSHLSGFKAAALTRT	304
<i>E. coli</i>	241	FVFSTTEKDGIGVEVALOWNDGFQENTYCFNNIPORDGGGTHLAGERAAMTRT	292
<i>N. gonorrhoeae</i>	244	FYAFGEKDGMSVECAMOWNDSYQESVOCFTNNIPORDGGGTHLTALROVMTRT	295
<i>S. aureus</i>	245	IYYDDESEGEIEVEIAMOATDELOGSIAHAFANNINTREGGTHLTGFKTALTRV	296
<i>B. subtilis</i>	245	IYIEGPKDGIITVEVALOWNDSYTSNTYSETNNINTYEGGTHEAGKTKGITEV	296
<i>M. xanthus</i>	305	LNSYAEKGS LWKDL-KETPTGEDAREGLSAVISVKLTNPFEGOTKTKLGNS	355
<i>E. coli</i>	293	LNAYMCKEGYSKK-AKVSATGDDAREGLIAVVSVKVPDPKFFSOTKDKLVSS	343
<i>N. gonorrhoeae</i>	296	INSYIEANEVAKK-AKVETAGDDMREGLTAVLSVKLPDPKFFSOTKDKLVSS	346
<i>S. aureus</i>	297	VNDYANSHDMLDLDGDNLRGEDVREGLTAVISVKHPDPKFFEGOTKTKLGNS	348
<i>B. subtilis</i>	297	INDYARKKGLIKE-NDPNLSGDDVREGLTAVISVKHPDPKFFEGOTKTKLGNS	347
<i>M. xanthus</i>	356	EVKGLVEQMVDNOLGSELEEFVMVAKKVAKIGDAARARLAARKARETTRRK	407
<i>E. coli</i>	344	EVKSAVEEQMNEILLAEYLLNENFDAKIVVGKIIDAAARAREARRAREMTRRK	395
<i>N. gonorrhoeae</i>	347	EIGPVVNEVINQALTDFFLEENENEAIIITGKIVDAARARCAARKAREITRRK	398
<i>S. aureus</i>	349	EVRGILVESVTHOQLGTFFFEENFDATAIISKAVEAARARCAAKQABELTRRK	400
<i>B. subtilis</i>	348	EARTITDTLTFSTAMETEMIEENFDAAKKIVDKGLMAARARMAAKKAREITRRK	399
<i>M. xanthus</i>	408	GVLDDGGGLPGKLADCCIRDPNESELYIVEGDSAGGS AKOGRDRNOAILPLR	459
<i>E. coli</i>	396	GALDLAGLPGLKADCCERDPALSELYIVEGDSAGGS AKOGRDRKNOAILPLK	447
<i>N. gonorrhoeae</i>	399	GLMDGLGLPGKLADCCERDPALSELYIVEGDSAGGS AKOGRDRKFOAILPLK	450
<i>S. aureus</i>	401	SAIESTSLPGKLADCCSRDPSESELYIVEGDSAGGS AKOGRDRKFOAILPLK	452
<i>B. subtilis</i>	400	SAIEISNLPGKLADCCSKDPSESELYIVEGDSAGGS AKOGRDRHFOAILPLR	451
<i>M. xanthus</i>	460	GKILNVEKL-LREMLTSAEIVTLITALGNGIGAEDYDFEKARYHRIFLMTDA	510
<i>E. coli</i>	448	GKILNVEKARFDMISSQEVATLITALGCGIGRDEYNPEKILRYHSIIIMTDA	499
<i>N. gonorrhoeae</i>	451	GKILNVA-----TLITALGAGIGKEEFNPEKILRYHRIIMTDA	488
<i>S. aureus</i>	453	GKILNVEKHRLDRITLDEIRALTITALGCGGIG-DEFDIEKARYHRLIIMTDA	503
<i>B. subtilis</i>	452	GKILNVEKARLDKILSNNEVRSMTITALGTGIG-EDFNIEKARYHKVVIIMTDA	502
<i>M. xanthus</i>	511	DVDGSHIRTLTLTSSSGRCRSCRRRAISTSRSSRSYKVTRNKDKDQYVKDEHA	562
<i>E. coli</i>	500	DVDGSHIRTLTLTFFYRQMPPIVERGHVYIAOPPLYKAKKQEQYIKDDEA	551
<i>N. gonorrhoeae</i>	489	DVDGAHIRTLTLTFFYRQMPPLVERGYIYIAOPPLYKAKYQKQERYLKDELE	540
<i>S. aureus</i>	504	DVDGAHIRTLTLTLLYRHRMPDLIEAGYVYAAOPPLYLVRYRGNTYDAMDEA	542
<i>B. subtilis</i>	503	DVDGAHIRTLTLTFFYRYMRQITENGYVYIAOPPLYKYOQGRVRYAYNDKE	554
<i>M. xanthus</i>	563	LNEYLLKIIASEHARVLT PGGE--LGGAELKALLEKVITYEERLEKQAKRRDA	612
<i>E. coli</i>	552	MDQYQISIALDGATLHTNASAPALAGEALEKLVSEYNATQKMINRMERRYPK	603
<i>N. gonorrhoeae</i>	541	KDQWLLGLALEKAKIVSDGRT--IEGAELADTAKQFLAKTVIEQESRFVDE	590
<i>S. aureus</i>	543	RDRIIEECNGNPTQ-----	542
<i>B. subtilis</i>	555	LEELLKTLPTQPKPG-----	557
<i>M. xanthus</i>	613	RVVDALVQGARLSAETLTGRGCPGGGGEGRLMTPSSAACRTCAACATSWCR	664
<i>E. coli</i>	604	AMLKELIYOPTL TEADLSDEQTVTRWVNALVSELNDKEQHG--SQWKFDVHT	653
<i>N. gonorrhoeae</i>	591	LVLRAMLHASPI---DLTSSSENADKAVAE LSGLLDEKEA-----LERIE	632
<i>S. aureus</i>	543	-----	549
<i>B. subtilis</i>	558	-----	569
<i>M. xanthus</i>	665	TPSTTPRSWCSTPTRMEPCGRRCSSTTPSCRRRSTWSCR LCAEHLRGAGOGAV	716
<i>E. coli</i>	654	NAEQNLFEPIVRVRTHGVDTDPLDHEFITGGEYRRRICTLGEKLRGLLEEDA	705
<i>N. gonorrhoeae</i>	633	GHEGHQFIK ITRKLHGNVMVSY-IEPKFLNSKAYQTLTQTAAALKGLVGEA	683
<i>S. aureus</i>	550	-----	570
<i>B. subtilis</i>	570	-----	569
<i>M. xanthus</i>	717	QGPRGRGEITVFSVQEVLA AVRKDAORGLGLORYKGLGEMNPEOLWDTTMMNF	768
<i>E. coli</i>	706	FIERGERROPVASFEQALDWLVKESRRGLSIO RYKGLGEMNPEOLWETTMDPF	757
<i>N. gonorrhoeae</i>	684	KLYKGNEYDADSFETALDILMSVAQKGMSTIO RYKGLGEMNPEOLWETTMDPF	735
<i>S. aureus</i>	571	-----VORLRYKGLGEMNPEOLWDTTMMNF	592
<i>B. subtilis</i>	570	-----IORYKGLGEMNATOLWETTMDPF	591
<i>M. xanthus</i>	769	ATRTLLQVRVEDAVESDEIFSLMGDAVEPRREFIERNALDVQNLDT	815
<i>E. coli</i>	758	ESRRMLRVTVEDAIADQLETTLMGDAVEPRRAFIERNALKAANIDT	804
<i>N. gonorrhoeae</i>	736	TVRRLLKVRTEDEIA-DEFEVTLMGDAVEPRRAFIERNALIAQNTIA	781
<i>S. aureus</i>	593	ENRVILKRTVEDAAAAADRMFNILMGDAVGPXQFLLKDEANDAEVVDI	639
<i>B. subtilis</i>	592	SSRRTLLQVTEEDAMDADETEEMLMGDAVEPRREFIERNARYVKNTDT	638

Fig. 3. Protein expression and Western blot analysis of *M. xanthus* 6-His-GyrB. (a) SDS-PAGE (10%) analysis of the *M. xanthus* 6-His-GyrB recombinant pPYGB expressed in *E. coli* strain M15(pREP4), without induction (lane 1) or with IPTG induction (lanes 2 and 3). (b) SDS-PAGE (10%) analysis of *E. coli* GyrB (lane 1) and *M. xanthus* 6-His-GyrB purified by affinity chromatography on novobiocin-Sepharose (lane 2) or on Ni-NTA (lane 3) columns. (c) Western blot analysis using polyclonal antibodies raised against the *E. coli* GyrB subunit. Lane 1, *E. coli* GyrB; lanes 2 and 3, *M. xanthus* 6-His-GyrB purified on novobiocin-Sepharose or on Ni-NTA resin, respectively. Molecular mass markers are indicated.

ATG codon, whereas a *Hind*III site was incorporated into the reverse primer, P2 (Fig. 1c). The PCR product was digested with *Bam*HI and *Hind*III and cloned into the vector pQE9. The resulting plasmid, pPYGB, was transformed into *E. coli* M15(pREP4). Ten colonies were screened for their ability to overexpress a fusion protein after induction with IPTG. The bacteria were lysed in sample buffer and their proteins were analysed using 10% SDS-PAGE (Laemmli, 1970). All the recombinant plasmids overexpressed a protein of approximately 90 kDa (Fig. 3a), in agreement with the calculated molecular mass of the deduced GyrB polypeptide including the six histidine residues.

Purification of the 6-His-GyrB fusion protein

The purification of the 6-His-GyrB fusion protein was achieved by either of two chromatographic procedures: (1) on Ni-NTA resin that specifically binds the six His residues and (2) on a novobiocin-Sepharose column that specifically binds DNA gyrase B (Orr & Staudenbauer, 1982; Staudenbauer & Orr, 1981). Total proteins were extracted from two 500 ml cultures of the over-expressing strain after induction with IPTG, followed by the purification procedures (see Methods). The eluted fractions were analysed by 10% SDS-PAGE (Laemmli, 1970). The results presented in Fig. 3(b) demonstrate that the GyrB protein was purified by both methods. The fractions contain the 90 kDa GyrB protein as well as smaller defined fragments of 40–50 kDa frequently resulting from a specific proteolysis of the GyrB protein (Orr *et al.*, 1984).

Plasmid pPYGB, overexpressing the *M. xanthus gyrB*, was transformed into the *E. coli gyrB* mutant LE316 that fails to grow at 42 °C (Orr *et al.*, 1979). The ability of *M.*

xanthus GyrB protein to complement the temperature-sensitive phenotype of the *E. coli gyrB* mutant was tested on LB agar and minimal medium plates at 32, 37 and 42 °C in the presence of different IPTG concentrations. The transformants failed to grow at the non-permissive temperature, indicating that the *E. coli gyrB* temperature-sensitive strain LE316 could not be complemented by the *M. xanthus* protein.

Immunological cross-reactivity

The proteins purified by the two types of chromatography were incubated with polyclonal antibodies raised against the *E. coli* GyrB subunit (Orr *et al.*, 1984). As shown in Fig. 3(c), the *M. xanthus* protein is recognized by the antibodies, indicating that it shares antigenic properties with the *E. coli* protein, and provides further evidence for our conclusion that the protein is the *M. xanthus* GyrB.

DISCUSSION

We cloned and determined the complete nucleotide sequence of the *gyrB* gene of *M. xanthus*. The protein is functionally and immunologically similar to its *E. coli* homologue as it binds novobiocin and cross-reacts with polyclonal antibodies raised against the *E. coli* protein.

The DNA sequence data predicted a protein of 815 amino acid residues with a calculated molecular mass of 89.63 kDa, which is consistent with the size of the recombinant protein expressed in *E. coli*. The gene contains a putative ribosome-binding site (AAGGA) and a potential transcription terminator downstream of the stop codon but lacks promoter sequences that resemble the TATAAT and TTGACA consensus

Fig. 2. Multiple alignment of gyrase B sequences from *M. xanthus* (this report), *E. coli*, *N. gonorrhoeae*, *S. aureus* and *B. subtilis*. Boxed letters indicate residues identical in more than three of the five organisms. The filled triangles indicate residues important in ATP binding and hydrolysis in *E. coli*. The open triangles indicate the two residues shown to be involved in coumarin resistance in *E. coli*.

sequences at -10 and -35, respectively. The absence of a consensus promoter, although intriguing, is not uncommon in myxobacteria. In addition, it may well be that the *M. xanthus gyrB* is the last gene, in an operon, transcribed from a longer polycistronic transcript. This situation would resemble that in other bacteria in which the organization of genes upstream of *gyrB* is highly conserved. The mapping of *gyrB* to the *oriC* region of the *M. xanthus* chromosome supports the idea that both its localization and organization are similar to those in other bacteria. Future analysis of our cosmid clones containing the *M. xanthus gyrB* region (pPYCA32, pPYCA40, pPYCA45 and pPYCA171) should reveal the detailed organization of genes around the *oriC* of *M. xanthus* and may be useful for the cloning of the *M. xanthus oriC*.

Comparison of the *M. xanthus* GyrB to that of other bacteria places it closer to the *E. coli* and *Neisseria gonorrhoeae* proteins (Fig. 2). The latter proteins have a non-conserved region of 170 amino acid residues near the C-terminus which is absent in other bacterial GyrB proteins such as *B. subtilis* GyrB, but present in *M. xanthus* GyrB (Fig. 2). There are several conserved regions, important for GyrB activity, which are present in all GyrB proteins, including the *M. xanthus* GyrB. These include the ATP- and novobiocin-binding domains, and the two consecutive glycines (at positions 220-221 in *E. coli* GyrB and 232-233 in *M. xanthus* GyrB) thought to form a flexible link between the two N-terminal subdomains (Gilbert & Maxwell, 1994; Fig. 2).

Although theoretically possible, it is unlikely that the cloned *M. xanthus* gene is a *parE* homologue for the following reasons: (1) the homology of the *M. xanthus* protein to other GyrB proteins is higher than its homology to ParE proteins and, moreover, the homology to other known GyrB proteins extends to the C-terminal region of the *M. xanthus* protein, which is missing from ParE proteins; (2) *parE* products are considerably smaller in size than GyrB proteins; and (3) ParE was not identified among novobiocin-binding proteins, or proteins which are recognized by the anti-GyrB antibodies.

Novobiocin inhibits non-competitively the binding of ATP to GyrB proteins. The binding site of this antibiotic, within the N-terminal fragment of the protein, has been identified in *E. coli* through the characterization of mutant GyrB proteins resistant to the antibiotic (Ali *et al.*, 1993). The fact that the *M. xanthus* GyrB strongly binds novobiocin, in a similar manner to other GyrB proteins, provides further evidence for their functional similarities. This conclusion is reinforced by the result that the *M. xanthus* protein is recognized by polyclonal antibodies raised against *E. coli* GyrB. The failure of the *M. xanthus* protein to complement the *E. coli* temperature-sensitive GyrB mutant LE316, at the non-permissive temperature, suggests that the *M. xanthus* 6-His-GyrB fusion protein may not be able to form a functional tetramer with the *E. coli* GyrA subunit *in vivo*. It is possible that the *M. xanthus* protein is not

functional at 42 °C, as *M. xanthus* does not grow at temperatures above 40 °C (Janssen *et al.*, 1977), or that the recombinant protein His-GyrB is inactive *in vivo*. The formation of a chimeric tetramer, although with poor activity, was previously demonstrated *in vitro* with the *B. subtilis* GyrB (Orr & Staudenbauer, 1982). However, such a reconstituted activity has never been tested *in vivo*.

Finally, the overexpression and purification of the *M. xanthus* GyrB protein should facilitate the study of its structure-function relationship; the data from such a study could be exploited in the screening and identification of new potent antimicrobial agents.

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