Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 333 (2005) 14–20
BBRC
www.elsevier.com/locate/ybbrc

Cloning and characterization of the chromosomal replication origin region of *Amycolatopsis mediterranei* U32

Yongqiang Tian [a], Pei Hao [b], Guoping Zhao [a,*], Zhongjun Qin [a,*]

[a] *Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, PR China*
[b] *Shanghai Center for Bioinformation Technology, 100 Qinzhou Road, Shanghai 200035, PR China*

Received 22 April 2005
Available online 26 May 2005

Abstract

The chromosomal replication origins (*oriC*) of gram positive, acid-fast actinomycetes have been investigated in streptomycetes and mycobacteria. A 1339 bp DNA fragment of the putative *oriC* region from the rifamycin SV producer *Amycolatopsis mediterranei* U32 was cloned by PCR amplification employing primers designed based on the conserved flanking genes of *dnaA* and *dnaN*. The 884 bp sequence of the intergenic region between *dnaA* and *dnaN* genes consists of 19 DnaA-boxes and two 13-mer AT-rich sequences, which is similar to the *oriC* structure of *Streptomyces lividans*. A mini-chromosome constructed by cloning the putative U32 *oriC* DNA fragment into an *Escherichia coli* plasmid was able to replicate autonomously, but was unstable, in *A. mediterranei* U32 with an estimated copy number of two per cell. Although efficient replication of the mini-chromosome in U32 requires the complete set of DnaA-boxes and AT-rich regions, only one of the AT-rich sequences together with part of the DnaA-boxes is sufficient, suggesting the presence of combinatorial alternatives for a functional *oriC* region of *A. mediterranei* U32. Phylogenetic analysis based on definite *oriC* sequences among eubacteria reflects well the relationship between these species.

Keywords: *Amycolatopsis mediterranei* U32; Chromosomal replication origin

Chromosomal replication origins (*oriC*) of *Escherichia coli* and other bacteria have been extensively investigated. Their locations are conserved within closely related bacteria, for example, between the *gidA* and *mioC* genes in enterobacteria [1,2] or between the *dnaA* and *dnaN* genes in actinomycetes [3,4]. The *oriC* regions usually contain both AT-rich sequences varying in length from 13 (e.g., *E. coli* [5] and *Pseudomonas* [6]) to 27 bp (e.g., *Bacillus subtilis* [7]) and several 9-mer DnaA protein recognition sequences (DnaA-boxes) [8]. It has been shown that the DnaA protein binds to the DnaA-boxes and initiates chromosomal replication at the AT-rich sequences, and then, double-strand DNA is unwound and the replication fork formed [9]. Both the in vivo genetic analysis and the in vitro biochemical studies about the interaction between the DnaA protein and the *oriC* region of *E. coli* have demonstrated that the *oriC* region is essential for chromosomal replication [5].

Actinomycetes are important among gram-positive prokaryotes for the pharmaceutical industry. Studies on the *oriC* regions of actinomycetes have been focusing on streptomycetes, which are the major sources of antibiotics [3,10], and mycobacteria, which are pathogens of some contagious diseases, e.g., tuberculosis [11]. The *Streptomyces lividans oriC* region contains two clusters of 19 DnaA-boxes and short but distributed AT-rich sequences [10], while the *oriC*s of mycobacteria consist of 3 or 5 DnaA-boxes and a single 11 nucleotide AT-rich sequence [4].

Amycolatopsis mediterranei U32 is a producer of rifamycin SV that has been widely used clinically in the

* Corresponding authors. Fax: +86 21 54924171 (Z. Qin).
E-mail addresses: gpzhao@sibs.ac.cn (G. Zhao), qin@iris.sipp.ac.cn (Z. Qin).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.05.070

treatment of tuberculosis, leprosy, and AIDS-related mycobacterial infection [12]. Due to their rare aerial hyphae and mycelium tending to lyse caused by their special cell wall structure, *Amycolatopsis* is morphologically and structurally different from *Streptomyces* and *Mycobacterium* [13], and it has been known to be genetically difficult for manipulation [14]. Thus, it will be interesting and important to study the differences and similarities of the *oriC* regions between *Amycolatopsis* and other actinomycetes' genera with respect to their structures and functions. In this study, we cloned and sequenced the *oriC* region of *A. mediterranei* U32. Characterization of the replication and inheritance property of a plasmid containing the *oriC* region in *A. mediterranei* U32 proved its function as a chromosomal replication origin. Finally, alternative reduced *oriC* regions sufficient for chromosomal DNA replication were identified.

Materials and methods

Bacterial strains, plasmids, culture conditions, and DNA manipulation. Escherichia coli strain DH5α and plasmid pUC18 were used as cloning host and plasmid. Culturing, transforming, and preparing plasmid DNA from *E. coli* and Southern blotting followed Sambrook and Russell [15]. *A. mediterranei* U32 (lab stock) were grown in Bennet broth as described previously [16]. Electrocompetent *A. mediterranei* was prepared and electroporation was carried out according to Ding et al. [17]. The transformants were selected and counted at 28 °C for 7 days on Bennet agar plates containing 30 μg/ml apramycin. An *E. coli–Amycolatopsis* shuttle plasmid pULVK2A, kindly provided by Professor J.F. Martin [18], was used as a positive control in transformation experiments. Chromosomal DNA from *A. mediterranei* U32 was prepared as described by Hopwood et al. [19].

Cloning, sequencing, and analyzing the oriC region of A. mediterranei. Intergenic region between *dnaA* and *dnaN* was cloned by a pair of primers P1: 5′-CGAATTCCGCCAGATCGCCATGTAC-3′ and P2: 5′-CGAATTCCGAGACCTCGTAGTCGAA-3′, which were designed by the conserved sequence in *dnaA* and *dnaN* [3,20]. Amplification reaction mixtures contained the following in a 20 μl volume: 10 mM KCl, 16 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 20 mM Tris–HCl, pH 8.8, 0.1% Triton X-100, 1 mg/ml BSA, 10% DMSO, and oligonucleotides at a final concentration of 1 μM. And 1.5 U high fidelity pfu polymerase (Sangon, Shanghai) was added. The following reaction conditions were used: 3 min heating at 95 °C followed by 30 cycles of 1 min at 94 °C, 45 s at 60 °C, and 1 min at 72 °C. DNA sequencing was performed in an Applied Biosystems 3730*xl* DNA Analyzer (Applied Biosystems Corp.). Oligonucleotide primers used in this study were synthesized in an ABI 3900 Synthesizer (Applied Biosystems Corp.). In order to detect putative DnaA-boxes, according to the 9-mer consensus sequence of DnaA-boxes of *S. lividans*, TTGTCCACA [10], find pattern set of GCG software package (the Apache Group) for finding conserved motifs in DNA sequences was used.

Determination of plasmid copy number. Amycolatopsis mediterranei U32 harboring the plasmid pTYQ9 which contains the *oriC* fragment was cultured, total chromosomal and plasmid DNA was isolated and digested by an appropriate DNA restriction enzyme, separated by electrophoresis, transferred to a nylon membrane, and hybridized by the ^{32}P-labeled 884 bp *oriC* DNA as a probe. Comparing the intensities of the chromosomal DNA band with that of the plasmid DNA band on an autoradiography film by adopting image analysis software AlphaEaseFC (Alpha Innotech corp.) can help estimate the copy number of the plasmid in the cell.

Analysis of the phylogenetic relationship based on the oriC sequence. Based on the present information about prokaryotes' *oriC*, only those with defined boundary between two characteristic genes (*dnaA* and *dnaN* in some cases while *gidA* and *mioC* in other cases) were retrieved from the NCBI GenBank database. The close vicinity nucleotides of these two characteristic genes were chosen as the boundaries of the *oriC* sequences. The 16S rDNA gene sequences from corresponding species were also retrieved from the GenBank database. Phylogenetic trees of either sequences were constructed based on the parsimony methodology (Phylip software on an internet server at Institut Pasteur, France) employing the corresponding sequence from the evolutionarily primitive eubacterial genus *Thermus* as the outgroup.

Results

The 884 bp dnaA/dnaN intergenic DNA fragment cloned from A. mediterranei U32 chromosome has all the sequence characteristics of bacterial oriC

In order to clone the *oriC* region of *A. mediterranei* U32, we assumed that it was situated between the *dnaA* and *dnaN* genes, which is the case among all the actinomycetes known so far [10,11]. A pair of oligonucleotide primers (Materials and methods) designed based on the conserved sequences of the *dnaA* and *dnaN* genes was used to PCR-amplify the chromosomal DNA of *A. mediterranei* U32, and a single 1.3 kb amplified DNA fragment was obtained and cloned into the *Eco*RI site of pUC18 for further sequencing analysis. This 1339 bp cloned fragment contained an 884 bp intergenic region between the *dnaA* and *dnaN* genes (Fig. 1). With-

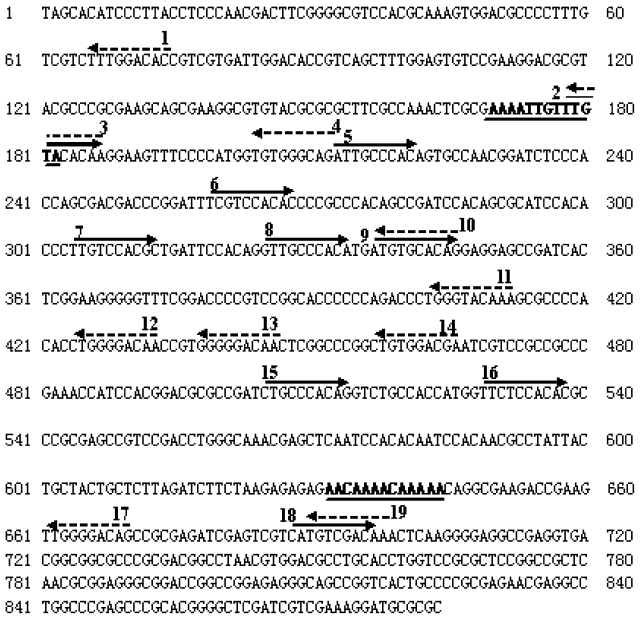

Fig. 1. DNA sequences and characterization of the spacer between the *dnaA* and *dnaN* genes of *A. mediterranei* U32. The predicted 19 *DnaA* boxes interspersing the 884 bp spacer are numbered and oriented by dash (backward) or solid (forward) arrows. Two AT-rich 13-mer sequences are indicated by underlines.

in this intergenic region, there were 19 DnaA-boxes with 9-mer consensus sequences, 5′-(T/C)TG(T/C)(C/G)C(A/C) CA-3′, and two 13-mer AT-rich sequences, AAAATTGTTTGTA and AACAAAACAAAAA (Fig. 1), which is similar to the structure of *oriC* of *S. lividans* [10]. These sequence characteristics suggested that the cloned 884 bp region contained the *oriC* of *A. mediterranei* U32.

Autonomous replication and unstable inheritance in A. mediterranei U32 of a plasmid containing the cloned oriC fragment proved its biological function

To investigate the replication activity of the cloned 884 bp DNA fragment, an *E. coli* plasmid/*Amycolatopsis* mini-chromosome, pTYQ9, was constructed by cloning the *A. mediterranei* U32 884 bp chromosomal fragment into an *E. coli* plasmid pTYQ2, a pUC18 derivative containing the apramycin-resistant gene. This plasmid was introduced into U32 by electroporation. Transformation efficiency detected by selecting apramycin-resistant colonies was 2.2×10^2/μg DNA. Although no plasmid DNA could be detected by direct agarose gel electrophoresis of the samples prepared from individual clones of the pTYQ9 transformants, apramycin-resistant transformants were obtained by introducing the same DNA preparation samples into electrocompetent *E. coli* DH5α cells. Plasmid DNAs of these *E. coli* transformants were digested with the restriction enzymes *Eco*RI or *Pst*I and were analyzed by agarose gel electrophoresis. The correct pTYQ9 DNA band pattern as expected (Fig. 2) suggested that no visible structural alterations occurred on pTYQ9 during its replication in strain U32.

Quantitation of the Southern blotting image employing the 884 bp probe against the total DNA preparations of U32 and the U32 carrying pTYQ9 indicated that there were only two copies of the *oriC*-containing mini-chromosome pTYQ9 in one cell of *A. mediterranei* U32 (Fig. 3).

The inheritance of pTYQ9 in *A. mediterranei* U32 was unstable. After 48 h incubation in Bennet broth without apramycin, about 60% of the cell population had lost the plasmid (Fig. 4).

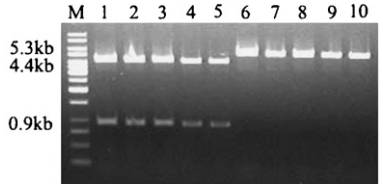

Fig. 2. Determination of the structure of plasmid pTYQ9 recovered from *A. mediterranei* U32. DNA was prepared from the strain U32 containing plasmid pTYQ9 and introduced into *E. coli* DH5α. Individual apramycin-resistant colonies were picked, plasmid DNA from them was digested by restriction enzymes *Eco*RI (lanes 2, 3, 4, and 5) and *Pst*I (lanes 7, 8, 9, and 10) and electrophoresed in 0.8% agarose gel at 130 V for 0.5 h. MBI 1 kb ladder (M), lanes 1 and 6 show plasmid pTYQ9 as control.

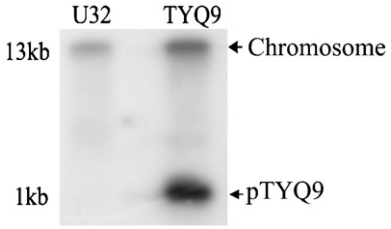

Fig. 3. Southern blot analysis of chromosomal *oriC* and plasmid carrying the *oriC* in *A. mediterranei* U32. Total DNA of strain U32 containing plasmid pTYQ9 was digested by *Eco*RI and electrophoresed in 0.8% agarose gel at 130 V for 1 h. The DNA in gel was transferred to nylon membrane and hybridized with ^{32}P-labeled 884 bp *oriC* fragment as probe. The *Eco*RI-digested DNA bands corresponding to the chromosome and plasmid are shown.

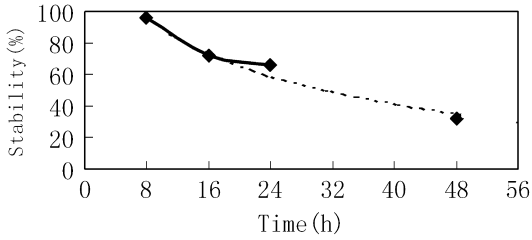

Fig. 4. Inheritance of plasmid pTYQ9 containing *oriC* in *A. mediterranei* U32. *Amycolatopsis mediterranei* U32 harboring the plasmid pTYQ9 was inoculated into Bennet broth without apramycin at 37 °C. At time points of 6, 12, 24, and 48 h, cells were harvested, diluted, and plated on Bennet agar with or without apramycin. Colonies from agar in the presence and absence of apramycin were counted and the ratio represented the stability (%) of inheritance of the plasmid.

Certain combinatorial alternatives of the cloned oriC region are sufficient for DNA replication in A. mediterranei U32, indicated by the serial deletion analysis

To determine the essential functional unit within the 884 bp *oriC* region of *A. mediterranei* U32 for primary DNA replication, serial deletions were made on the region and the resulting DNA fragments were cloned into the *Eco*RI site of pTYQ2 to generate a series of plasmids/mini-chromosomes, pTYQ14, pTYQ15, pTYQ16, and pTYQ17 (Fig. 5). These plasmids were introduced into U32 cells by electroporation, and the number of apramycin-resistant transformants was counted to measure the efficiency of replication of the plasmids in U32.

As shown in Fig. 5, plasmid pTYQ14, a shorter derivative of pTYQ9, which contained almost the complete set of DnaA-boxes except the 1st one and the two AT-rich regions but deleted all the flanking regions in the vicinity to *dnaA* and *dnaN*, had high transformation efficiency as that of pTYQ9 (145 and 220 transformants/μg DNA, respectively). Both plasmid pTYQ16 containing the region between the 1st and the 17th DnaA-box,

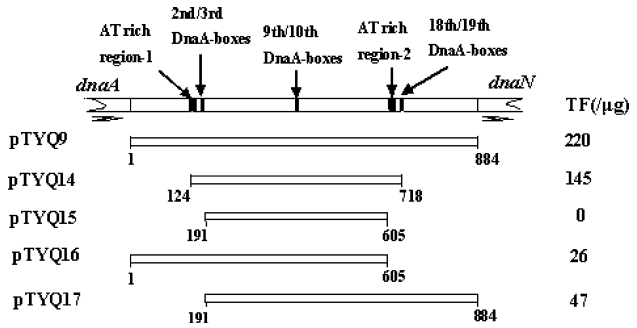

Fig. 5. Identification of the essential regions for replication employing the plasmids containing different lengths of the *oriC* in *A. mediterranei* U32. The oligonucleotides used for PCR amplifying different lengths of *oriC* include: T1 (5′-GGAATTCTAGCACATCCCTTACCTTC-3′), T2 (5′-GGAATTCTGTACGCGCGCTTCGCCAA-3′), T3 (5′-GGAATTCAGTTTCCCCATGGTGGGCA-3′), T4 (5′-GGAATTCCTAAGAGCAGTAGCAGTAA-3′), T5 (5′-GGAATTCCTACCTCGGCCTCCCCTTG-3′), and T6 (5′-GGAATTCGCGCGCATCCTTTCGACGA-3′). The PCR-amplified products from the pairs of primers T1 + T6, T2 + T5, T3 + T4, T1 + T4, and T3 + T6 were treated with *Eco*RI and ligated into plasmid pTYQ2 to obtain plasmids pTYQ9, pTYQ14, pTYQ15, pTYQ16, and pTYQ17, respectively. The transformation frequency (TF) in strain U32 of these plasmids is shown. The numbers under the bar are start and end nucleotides of the *oriC* fragment.

including AT-rich region-1, and plasmid pTYQ17 containing the region between the 4th DnaA-box and the 18th/19th DnaA-box pair including AT-rich region-2, showed a lower transformation frequency (26 and 47 transformants/μg DNA, respectively) than the above two plasmids. No apramycin-resistant transformant could be obtained when plasmid pTYQ15 was introduced into strain U32, which contained the common region of plasmids pTYQ16 and pTYQ17 (no AT-rich region and with DnaA-boxes from the 4th to the 17th). It thus suggested that although alternative combination of one AT-rich region plus a few DnaA-boxes was sufficient for DNA replication in *A. mediterranei* U32, efficient chromosomal DNA replication required the complete set of DnaA-boxes and two AT-rich regions.

Phylogenetic relationship among eubacteria based on their oriC sequences with clear boundaries matches well with that based on the 16S rDNA sequence analysis

The known *oriC* sequences of eubacteria can be categorized into three groups based on the differences of sequence boundaries of these fragments. In the first group, *oriC* is located between *dnaA* and *dnaN*. Actinomycetes as well as the evolutionarily primitive eubacterial genus *Thermus* bear the same feature [3,4,21]. Similarly, the *oriC*s of the second group are located between *gidA* and *mioC*. The gram-negative facultative anaerobic enteric bacteria, such as *Escherichia*, *Enterbacter*, *Erwinia*, *Shigella*, *Samonella*, *Yersinia*, and *Klebsiella*, as well as the related genus *Vibrio*, belong to this group [1,2,22]. The characteristics of the third group *oriC* are very different from those of the other two. It is separated by *dnaA* which is composed of the upstream and the downstream regions of *dnaA* (but upstream of *dnaN*). This kind of *oriC* has been recognized in some gram-positive genera, such as *Bacillus* and *Mycoplasma* [7,23]. Due to the limited information available, it is still difficult to define the boundaries of the *oriC* sequences of the third group. Therefore, only the *oriC* sequences of the first two groups with unambiguous boundaries were used for phylogenetic analysis. We chose the evolutionarily primitive eubacterial genus *Thermus* as the outer group and the *oriC* sequence-based parsimony phylogenetic tree (Fig. 6A) was compared to that of the 16S rRNA gene-based tree (Fig. 6B). It is obvious that these two phylogenetic trees illustrated very similar trends of evolution. In particular, both the trees presented two identical clusters of species. For the sake of illustration, they are designated Groups I and II (Fig. 6). Group I contains the genera of the *Enterobacteriaceae* family and *Vibrio*, while Group II mainly contains species of high GC-content actinomycetes, including the genera of *Mycobacterium*, *Streptomyces*, and *Amycolatopsis*. The clustering of these two groups matches well with the characteristic differences in the flanking genes of their respective *oriC* sequences.

Discussion

Chromosomal replication origin is a *cis*-acting replicator, which controls the initiation of chromosome replication via binding a *trans*-acting protein, DnaA, that unwinds the multiple DnaA-boxes and AT-rich sequences [24]. The structure and function of *oriC* from various bacteria were investigated [6,8,10]. In this report, we showed that *A. mediterranei* U32 bore the characteristic structure of *dnaA*-DnaA-boxes/AT-rich sequences-*dnaN* in its *oriC* region, with a lower G + C% feature of other *oriC* sequences [10,11].

The consensus sequence of 19 DnaA-boxes of the *A. mediterranei oriC* region is (T/G)TG(T/C)(C/G)C(A/C)CA. It is similar to (T/C)(T/C)(G/A/C)TCCACA, the DnaA-box motif of *S. lividans*; but shows more variability at positions 1 and 5. Similar to the 9-mer sequence character of the DnaA-boxes of *S. lividans*, the third nucleotide, G, and the eighth nucleotide, C, are highly conservative (Table 1). The third nucleotide G/C reflects high G + C content of actinomycetes [24], whereas the nucleotide of the same site is generally A in other eubacteria [5,7]. Compared with the compositions of DnaA-boxes from three actinomycetes (*A. mediterranei*, *S. lividans*, and *Mycobacterium leprae*), the consensus sequences of three genera are similar, but

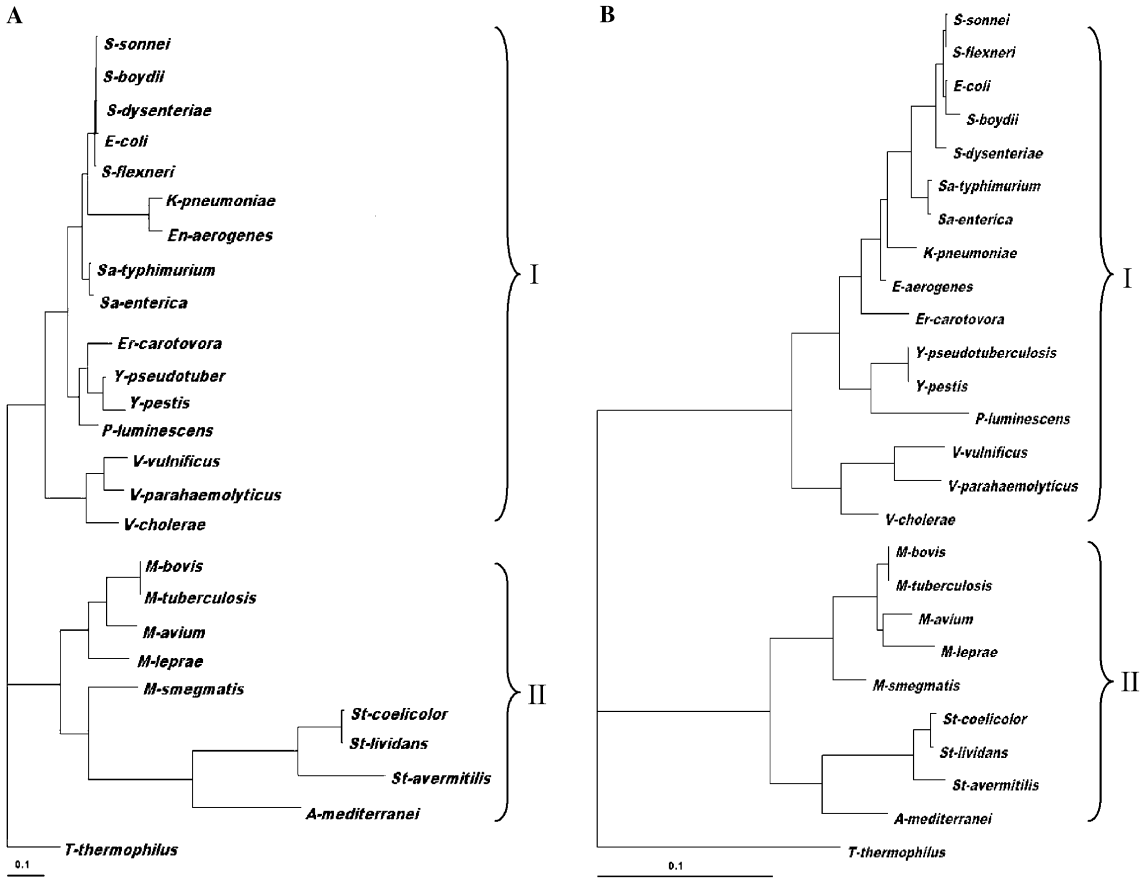

Fig. 6. Comparison of the phylogenetic tree based on the sequences of *oriC* and the tree based 16S rRNA gene. The tree was drawn by Phylip 3.6a with the parsimony method. The *oriC* sequences of strains are obtained from the NCBI GenBank database. (A) The phylogenetic tree based on oriC sequences and (B) the phylogenetic tree based on 16S rRNA genes. *S*: *Shigella*; *E*: *Escherichia*; *K*: *Klebsiella*; *En*: *Enterobacter*; *Sa*: *Salmonella*; *Er*: *Erwinia*; *Y*: *Yersinia*; *P*: *Photorhabdus*; *V*: *Vibrio*; *M*: *Mycobacterium*; *St*: *Streptomyces*; *A*: *Amycolatopsis*; *T*: *Thermus*.

Table 1
The frequency of nucleotide usage of the 9-mer consensus sequences in the 19 DnaA-boxes of *A. mediterranei* U32

Nucleotide	The position no. of DnaA-boxes								
	1	2	3	4	5	6	7	8	9
A	11	0	0	0	11	0	**79**	5	**89**
T	**63**	**89**	0	**79**	0	5	0	0	0
G	5	11	**95**	0	16	11	0	0	5
C	21	0	5	21	**73**	**84**	21	**95**	5

The bold numbers are the highest frequency of used-nucleotides.

the number and composition of the DnaA-boxes are diverse (Table 2).

After binding at the DnaA-boxes by the DnaA protein, unwinding at the AT-rich sequences is a crucial step for the initiation of chromosomal replication. The AT-rich regions exist in the *oriC* region of prokaryotes [24] and eukaryotes widely [25], and do not share consensus sequences [5,7,10]. A hybrid origin with the main part from *oriC* of *E. coli* and the AT-rich region from *B. subtilis* could be unwound by *E. coli* DnaA [26] suggests that the structures vs. sequences of the *oriC* are important for the initiation of replication. Our data show that efficient chromosomal DNA replication of *A. mediterranei* U32 required the full 19 DnaA-boxes and two AT-rich regions. However, one of the AT-rich sequences and part of the DnaA-boxes are sufficient for chromosomal DNA replication in *A. mediterranei* U32, suggesting the presence of alternative combination of *oriC* elements for the initiation of chromosomal replication.

Unstable inheritance of the plasmid containing the *A. mediterranei oriC* region is not unique. Similar phenomena have also been observed in other actinomycete species [27]. The low copy number and unstability of the *oriC* plasmids could be caused by competing for limited DnaA proteins by the same replicon of chromosome [27].

Table 2
Comparison of the conservation of nucleotides in DnaA-boxes of *oriC* among *A. mediterranei*, *S. lividans*, and *M. leprae*

The number of base of DnaA-box	1	2	3	4	5	6	7	8	9	Number of DnaA-boxes	Length of *oriC* (bp)
nt in consensus sequence	T	T	G	T	C	C	A	C	A		
Conservation (%)											
A. mediterranei	63	89	95	79	73	84	79	95	89	19	884
S. lividans	74	79	53	95	89	100	95	100	68	19	934
M. leprae	100	100	50	100	100	75	100	75	100	4	521

Note. The GenBank Accession Nos. are AY606113, AF077942, and Z70722, respectively.

Although *oriC* is an essential functional element for cell duplication, Yoshikawa and Ogasawara [8] suggested that the sequences of *oriC* regions were conserved only among closely related organisms. Our phylogenetic analysis employing *oriC* sequences with well-defined boundaries(*dnaA–dnaN* and *gidA–mioC*) indicated that the phylogenetic relationship among remote relative genera is comparable with that based on the 16S rRNA gene sequences (Fig. 6). Thus, these *oriC* sequences offered one more functional molecular marker, which can be used for general phylogenetic analysis. Of course, another group of *oriC* with *B. subtilis* kind of characteristics was not included in this study due to its ambiguous boundaries. It suggests that further detailed analysis of this kind of *oriC* is of great importance.

Our data also indicated that phylogenetically, *A. mediterranei* is closer to *S. lividans* than to *M. leprae* based on either *oriC* sequences or 16S rRNA sequences. This is consistent with the phylogenetic analysis of other studies [28].

Studying the characterization of the chromosomal origin of replication can help us to learn about the mechanism of the initiation of replication, and also help us to construct a genetic manipulation system in plasmidless strains by mini-chromosome containing *oriC* region [23]. On the other hand, as an important functional unit, the change of nucleotides in this region could reflect the phylogenic relationship between bacterial strains. The tree based on *oriC* sequences from GenBank gave the consensus result of the 16S rRNA gene among closely related organisms (Fig. 6).

Acknowledgments

We thank Professor J.F. Martin for plasmid pULV-K2A, and Hao Xu the Bioinformation Center, Shanghai Institute for Biological Sciences, for *oriC* sequence collection. These investigations were supported by grants from The National Natural Science Foundation of China (30325003) and "863" Project (2002AA227021) to Z. Qin.

References

[1] M. Meijer, E. Beck, F. Hansen, H. Bergmans, W. Messer, K. von Meyenburg, H. Schaller, Nucleotide sequence of the origin of replication of the *Escherichia coli* K-12 chromosome, Proc. Natl. Acad. Sci. USA 2 (1979) 580–584.
[2] J. Cleary, D. Smith, N. Harding, J. Zyskind, Primary structure of the chromosomal origins (oriC) of *Enterobacter aerogenes* and *Klebsiella pneumoniae*: comparisons and evolutionary relationships, J. Bacteriol. 150 (1982) 1467–1471.
[3] J. Zakrzewska-Czerwinska, H. Schrempf, Characterization of an autonomously replicating region from the *Streptomyces lividans* chromosome, J. Bacteriol. 174 (1992) 2688–2693.
[4] M.H. Qin, M.V.V.S. Madiraju, S. Zachariah, M. Rajagopalan, Characterization of the *oriC* region of *Mycobacterium smegmatis*, J. Bacteriol. 179 (1997) 6311–6317.
[5] A. Kornberg, T.A. Baker, DNA Replication, W.H. Freeman and Company, New York, 1992.
[6] D.W. Smith, T.W. Yee, C. Baird, V. Krishnapillai, Pseudomonad replication origins: a paradigm for bacterial origins? Mol. Microbiol. 5 (1991) 2581–2587.
[7] H. Yoshikawa, R.G. Wake, Initiation and termination of chromosome replication, in: A.L. Sonenshein, J.A. Hoch, R. Losick (Eds.), *Bacillus Subtilis* and Other Gram-positive Bacteria: Biochemistry, Physiology and Molecular Genetics, ASM, Washing DC, 1993, pp. 507–528.
[8] H. Yoshikawa, N. Ogasawara, Structure and function of DnaA and the DnaA-box in eubacteria: evolutionary relationships of bacterial replication origins, Mol. Microbiol. 5 (1991) 2589–2597.
[9] K. Sekimizu, D. Bramhill, A. kornberg, ATP activates dnaA protein in initiating replication of plasmids bearing the origin of the *E. coli* chromosome, Cell 50 (1987) 259–265.
[10] D. Jakimowicz, J. Majka, W. messer, C. Speck, M. Fernandez, M.C. Martin, J. Sanchez, F. Schauwecher, U. Keller, H. Schrempf, J. Zakrzewska-Czerwinska, Structural elements of the *Streptomyces oriC* region and their interactions with the DnaA protein, Microbiology 144 (1998) 1281–1290.
[11] L. Salazar, H. Fsihi, E. de Rossi, G. Riccardi, C. Rios, S. Cole, H. Takiff, Organization of the origins of replication of the chromosomes of *Mycobacterium smegmatis*, *Mycobacterium leprae* and *Mycobacterium tuberculosis* and isolation of a functional origin from *M. smegmatis*, Mol. Microbiol. 20 (1996) 283–293.
[12] K.A. Sepkowitz, J. Rafalli, L. Riley, T.E. Kiehn, D. Armstrong, Tuberculosis in the AIDs era, Clin. Microbiol. Rev. 8 (1995) 180–199.
[13] M.P. Lechevalier, H. Prauser, D.P. Labeda, J.-S. Ruan, Two new genera of nocardioform actinomycetes: Amycolata gen. nov. and Amycolatopsis gert. nov., Int. J. Syst. Bacteriol. 36 (1986) 29–37.
[14] T. Schupp, M. Divers, Protoplast preparation and regeneration in *Nocardia mediterranei*, FEMS Microbiol. Lett. 36 (1986) 159–162.
[15] J. Sambrook, D. Russell, Molecular Cloning: a Laboratory Manual, third ed., Cold Spring Harbor Laboratory Press, USA, 2001.
[16] A. Mejia, J. Barrios-Gonzalez, G. Viniegra-Gonzalez, Overproduction of rifamycin B by *Amycolatopsis mediterranei* and its relationship with the toxic effect of barbital on growth, J. Antibiot. 51 (1998) 58–63.

[17] X. Ding, Y. Tian, J. Chiao, G. Zhao, W. Jiang, Stability of plasmid pA387 derivatives in *Amycolatopsis mediterranei* producing rifamycin, Biotechnol. Lett. 25 (2003) 1647–1652.
[18] C.V. Kumar, J.J.R. Coque, J.F. Martin, Efficient transformation of the cephamycin C producer *Nocardia lactaamdurans* and development of shuttle and promoter-probe cloning vectors, Appl. Environ. Microbiol. 60 (1994) 4086–4093.
[19] T. Kieser, M.J. Bibb, M.J. Buttner, K.F. Chater, D.A. Hopwood, Practical *Streptomyces* Genetics, The John Inns Foundation, Norwich, England, 2000.
[20] K. Eiglmeier, N. Honore, S.A. Woods, B. Caudron, S.T. Cole, Use of an ordered cosmid library to deduce the genomic organization of *Mycobacterium leprae*, Mol. Microbiol. 7 (1993) 197–206.
[21] S. Schaper, J. Nardmann, G. Luder, R. Lurz, C. Speck, W. Messer, Identification of the chromosomal replication origin from *Thermus thermophilus* and its interaction with the replication initiator DnaA, J. Mol. Biol. 299 (2000) 655–665.
[22] A. Saha, S. Haralaka, R. Bhadra, A naturally occurring point mutation in the 13-mer R repeat affects the oriC function of the large chromosome of *Vibrio cholerae* O1 classical biotype, Arch. Microbiol. 182 (2004) 421–427.
[23] C. Cordova, C. Lartigue, P. Sirand-Pugnet, J. Renaudin, R. Cunha, A. Blanchard, Identification of the origin of replication of the *Mycoplasma pulmonis* chromosome and its use in oriC replicative plasmids, J. Bacteriol. 184 (2002) 5426–5435.
[24] W. Messer, The bacterial replication initiator DnaA. DnaA and *oriC*, the bacterial mode to initiate DNA replication, FEMS. Microbiol. Rev. 26 (2002) 355–374.
[25] M. Mechali, DNA replication origins: from sequence specificity to epigenetics, Nat. Rev. Genet. 2 (2001) 640–645.
[26] H. Seitz, M. Welzeck, W. Messer, A hybrid bacterial replication origin, EMBO Rep. 2 (2001) 1003–1006.
[27] J. Zakrzewska-Czerwinska, J. Majka, H. Schrempf, Minimal requirements of the *Streptomyces lividans* 66 oriC region and its transcriptional and translational activities, J. Bacteriol. 177 (1995) 4765–4771.
[28] A. Yokota, Phylogenetic relationship of actinomycetes, in: The Society for Actinomycetes Japan, Atlas of Actinomycetes, Asakura Publishing Co. Ltd 1997, pp. 194–197.