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Prevalence and distribution of nucleotide sequences typical for pMEA-like accessory genetic elements in the genus *Amycolatopsis*

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Abstract

The prevalence and distribution of pMEA-like elements in the genus *Amycolatopsis* was studied. For this purpose, a set of 95 recently isolated *Amycolatopsis* strains and 16 *Amycolatopsis* type strains were examined for the presence of two unique pMEA-sequences (*repAM* and *traJ*), encoding proteins essential for replication and conjugative transfer. Homologues of *repAM* and *traJ* were found in 10 and 26 of 111 investigated strains, respectively, a result which shows that pMEA-like sequences, though not very abundant, can be found in several *Amycolatopsis* strains. Phylogenetic analysis of the deduced RepAM and TraJ protein sequences revealed clustering with the protein sequences of either pMEA300 or pMEA100. Furthermore, two geographically different populations of pMEA-like elements were distinguished, one originating in Europe and the other in Australia and Asia. Linkage between the distribution of *repAM* and *traJ* and the chromosomal identifier, the 16S rRNA gene, indicated that these elements coevolved with their hosts, suggesting that they evolved in an integrated form rather than by horizontal gene transfer of the free replicating form.

Introduction

The order *Actinomycetales* encompasses Gram-positive, mycelium-forming bacteria, many of which are capable of producing antibiotics and other useful secondary metabolites for medical and agricultural applications (Bérdy, 2005). Accessory genetic elements, such as plasmids and integrative elements, are common in actinomycetes (Grohmann *et al.*, 2003).

The actinomycete genus *Amycolatopsis* was proposed by Lechevalier *et al.* (1986) and currently contains 34 validly described species which form a distinct phylogenetic line within the evolutionary branching encompassed by the family *Pseudonocardiaceae* (Carlsohn *et al.*, 2007). However, recently Tan *et al.* (2006) characterized several novel *Amycolatopsis* isolates and in doing so showed that species diversity within the genus is much higher than previously thought. *Amycolatopsis* strains are a rich source of antibiotics and other secondary metabolites; well known examples are vancomycin produced by *Amycolatopsis orientalis* (Pittenger & Brigham, 1956), an antibiotic that is currently the last resort against methicillin-resistant *Staphylococcus aureus*, and rifamycin, which is produced by *Amycolatopsis mediterranei* (Sensi *et al.*, 1959) and used to fight tuberculosis and leprosy. Further exploration of the genus *Amycolatopsis* is needed in the search for new antibiotics. To this end, identification and characterization of mobile genetic elements of *Amycolatopsis* strains is important for the development of new cloning, expression, and shuttle vectors, and to

Fig. 1. Map of pMEA300 (13.3 kb) of *Amycolatopsis methanolica*. The genes *repAM*, *xis*, *int* and *traJ* are shown as black arrows and the *attP* site as a black bar. The other *orf*s are depicted as grey arrows.

gain an insight into the origin, evolution, and functional properties of these extrachromosomal elements.

The integrative elements of *Amycolatopsis methanolica* and *A. mediterranei*, pMEA300 (13.3 kb) and pMEA100 (23.3 kb), respectively, have been sequenced (Vrijbloed, 1996; E.M. te Poele *et al*. unpublished data). Characterization of deletion derivatives of pMEA300 allowed the identification of genes required for replication, regulation, integration and conjugation (Vrijbloed *et al*., 1994, 1995a–c) (Fig. 1). Based on structural and functional similarities, both pMEA300 and pMEA100 have been classified as integrative and conjugative elements (ICE) together with elements of several other actinomycetes (Raynal *et al*., 1998) such as SLP1 from '*Streptomyces coelicolor*' A3(2) (Bibb *et al*., 1981), pSAM2 from *Streptomyces ambofaciens* (Pernodet *et al*., 1984), pIJ110 from *Streptomyces parvulus* (Hopwood *et al*., 1984), pIJ408 from *Streptomyces glaucescens* (Hopwood *et al*., 1984; Sosio *et al*., 1989), pSG1 from *Streptomyces griseus* (Cohen *et al*., 1985), pSE101 (Brown *et al*., 1988) and pSE211 (Brown *et al*., 1990) from *Saccharopolyspora erythraea*, and probably pMR2, a plasmid from *Micromonospora rosaria* that has recently been sequenced (Hosted Jr *et al*., 2005). This class of elements integrates site-specifically in a tRNA gene of the host genome, and the majority of these elements can also replicate autonomously and be transferred to other strains through conjugation. The integrase (Int) directs site-specific DNA recombination between the *attP* site on the element and a chromosomal *attB* site (Boccard *et al*., 1989).

The organization of genes involved in replication, excision and integration (*repAM*, *xis*, *int* and *attP*) in pMEA300 (Fig. 1) is conserved and appears to be a general feature of the actinomycete ICE class. Most of these elements can mediate the pock-formation phenotype, reflecting growth retardation of the recipient which occurs upon conjugation (Vrijbloed *et al*., 1995c). Deletion studies have revealed that the *traJ* gene of pMEA300 is essential for transfer of the element into recipient strains lacking pMEA300 (Vrijbloed *et al*., 1995c). TraJ of pMEA300 displays a high similarity to TraJ of pMEA100 (E.M. te Poele *et al*., unpublished data) (33% identity) and to a putative plasmid transfer protein of *Streptomyces tenjimariensis* (GenBank accession no. CAH60136) (29% identity). The C-terminal part of TraJ contains an FtsK/SpoIIIE domain and TraJ shows 27% identity to a cell division FtsK/SpoIIIE protein of *Frankia* sp. EAN1pec (GenBank accession no. ZP_00571168) and 27% identity to TraB of *Streptomyces ghanaensis* plasmid pSG5 (GenBank accession no. YP_001004136). It has been shown that this latter protein is a septal DNA translocator that mediates a unique conjugation mechanism able to translocate unprocessed double-stranded DNA molecules to recipient strains (Reuther *et al*., 2006).

The replication initiator protein RepAM of pMEA300 and its homologues in pMEA100 ($RepAM_{pMEA100}$) and pSE211 ($RepAM_{pSE211}$) form a novel class of replication initiator proteins (te Poele *et al*., 2006). The amino acid sequences of these proteins do not display similarity to previously known replication proteins, but are similar to a prophage-like protein of *Mycobacterium tuberculosis* bacteriophage ϕRv2 (Hendrix *et al*., 1999). Binding studies with purified RepAM protein revealed that it is able to bind to multiple identical 8-bp repeats within its own *repAM* coding sequence (te Poele *et al*., 2006). The repeat sequences are able to form a stable secondary structure. Similar structures with multiple identical 8-bp inverted repeats have been found at the 3′ end of the putative replication initiator genes of pMEA100 ($repAM_{pMEA100}$) and pSE211 ($repAM_{pSE211}$).

Because of the unique characteristics and novelty of these replication initiator and transfer proteins, we are interested in the prevalence and distribution of this new class of pMEA-like elements amongst members of the genus *Amycolatopsis*. In the present study ~100 recently isolated *Amycolatopsis* strains (Tan *et al*., 2006) were examined for the presence of two unique pMEA-sequences, namely *repAM* and *traJ*. The molecular systematics dataset generated for a large number of *Amycolatopsis* strains provides a very interesting biogeographical profile of the evolution and distribution of pMEA-like elements.

Materials and methods

Bacterial strains, integrative elements and culture conditions

Amycolatopsis methanolica NCIB 11946^T (de Boer *et al*., 1990) was grown on Trypticase Soy Broth (TSB) (BBL Microbiology Systems, Cockeysville, MD) plates. The pMEA100-containing strain *A. mediterranei* DSM 43304 was cultivated on plates of SM3 medium (Tan *et al*., 2006) omitting the antibiotics, whereas the pSE211-containing strain *Sac. erythraea* DSM 40517 was cultivated on GYM (Ochi, 1987). The 111 *Amycolatopsis* test strains included 15 *Amycolatopsis* type strains, the closely related *Sac. erythraea* and *Amycolatopsis* strains which were isolated from soil

samples taken from Leazes Park, Newcastle upon Tyne, UK, and from several locations in Australia, i.e. soils from Ayers Rock, Kings Canyon, Marla, Port Augusta, Alice Springs, Ormiston Gorge, Simpsons Gap, and from dry wash away soils from Coopers Creek, Lake Hope and Mulka. The type strains *A. methanolica*, *A. orientalis*, *A. sacchari* and *Sac. erythraea* were isolated in Asia (Pittenger & Brigham, 1956; de Boer *et al*., 1990; Goodfellow *et al*., 2001; Carreras *et al*., 2002), and *A. mediterranei* came from the South coast of France (Margalith & Beretta, 1960). The organisms were cultivated on TSB plates for 1–8 days at 30 °C. *Escherichia coli* DH5α, JM109 and TOP10 cells (Invitrogen, Groningen, The Netherlands) were grown at 37 °C on Luria–Bertani medium supplemented with 50 μg mL^{-1} ampicillin.

DNA manipulations

Total genomic DNA of the *Amycolatopsis* strains was isolated from 2-mL cultures using the Wizard Genomic DNA Purification Kit (Promega Benelux B.V., Leiden, The Netherlands). The DNA preparations were dissolved overnight at 4 °C in 50 μL Nuclease-Free Water (Ambion, Inc., Austin, TX). Whole-cell lysates were obtained by resuspending *Amycolatopsis* colony material in 20 μL Nuclease-Free Water with subsequent boiling for 10 min. Plasmids from *Amycolatopsis methanolica* NCIB 11946^T and *Amycolatopsis* strain GY027 were isolated using the Qiagen Plasmid Midi Kit (Qiagen, Venlo, The Netherlands) with some modifications, as described by te Poele *et al*. (2006). Plasmid extraction from the *E. coli* strain was performed using the QIAprep Spin Miniprep Kit (Qiagen).

PCR amplification

PCR-based analysis was performed on either whole-cell lysates or total genomic DNA. PCR primers were designed to conserved regions of the aligned sequences of *repAM* ($repAM_{pMEA300}$, $repAM_{pMEA100}$ and $repAM_{pSE211}$) and of the *traJ* genes ($traJ_{pMEA300}$ and $traJ_{pMEA100}$). The primers repAMF (5′-CACATGCGNCGCCGCGCCNCCGG-3′) and repAMR (5′-GTAGCGGNTCCAGGCGTCG-3′) amplified a 550-bp fragment of *repAM* that is located upstream of the RepAM binding region (te Poele *et al*., 2006). The PCR products of pMEA300 and pMEA100 showed 45% DNA identity and 35% amino acid identity, and the products of pMEA300 and pSE211 were 71% identical at the DNA level and 62% identical at the amino acid level. The identity between the *repAM* PCR products of pMEA100 and pSE211 was 58% at the DNA level and 39% at the amino acid level. PCR primers traJF (5′-AAGCGGTGGCGCGACAC-3′) and traJR (5′-CAGGAACACCCCGTAGGTGA-3′) were used to amplify a 330-bp region of *traJ*. The pMEA300 and pMEA100 *traJ* PCR products showed 73% identity at the DNA sequence level and 66% identity at the amino acid level. The *repAM* and *traJ* amplified regions were checked against the NCBI database to confirm that the sequences were unique and specific for the pMEA-like elements. Oligonucleotide primers B8F (5′-AGAGTTTGATCMTGGCTCAG-3′) (Edwards *et al*., 1989) and U1406R (5′-ACGG GCGGTGTGTRC-3′) (Lane, 1991) were used to amplify the 16S rRNA gene. Reaction mixtures (final volume of 50 μL) for the PCR amplifications of *repAM* (repAMF and repAMR) or *traJ* (traJF and traJR) or for the 16S rRNA gene (B8F and U1406R) contained 1.5 mm $MgCl_2$, 10% dimethylsulphoxide, 0.2 mg mL^{-1} bovine serum albumin, 0.2 mm deoxynucleoside triphosphates, 0.4 μm of each primer, 0.04 U *Taq* polymerase (Amersham Biosciences, Roosendaal, The Netherlands) with the appropriate reaction buffer and 200 ng template DNA or 1 μL whole cell lysate. The amplification program was as follows: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 62 °C (*repAM*) or 58 °C (*traJ*) or 49 °C (16S rRNA gene) for 30 s, 72 °C for 33 s (*repAM*) or 20 s (*traJ*) or 1 min 25 s (16S rRNA gene). The last cycle was followed by a final extension step of 5 min (*repAM* and *traJ*) or 7 min (16S rRNA gene) at 72 °C. Amplification products were analyzed by gel electrophoresis in agarose gels (1.0% or 1.5% w/v) stained with ethidium bromide; SmartLadder (Eurogentec, Seraing, Belgium) was used as molecular weight marker. PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems, Nieuwekerk a/d IJssel, The Netherlands).

Southern hybridization

Southern hybridization was performed using a DIG DNA Labeling and Detection Kit (Roche Diagnostics, Almere, The Netherlands). EcoRI-digested genomic DNA (∼200–500 ng) of the *Amycolatopsis* strains was applied to a positively charged membrane (Hybond-N, Amersham Biosciences) using a 48-well slot blot apparatus (Hoefer Scientific, San Francisco, CA). For construction of the pMEA-specific probes, *repAM* and *traJ* fragments were amplified from pMEA300 using the primers repAMF/R and traJF/R. The PCR products were cloned into the pGEM-T easy vector (Promega) and one clone for each probe was used as template for probe amplification by PCR and subsequent DIG-labeling. Sequencing of these two clones confirmed that they contained the correct insert without amplification errors. Both probes were hybridized at 65 °C. After hybridization with the first probe, the blot was stripped (incubation in 2 × SSC for 5 min, 0.4 m NaOH for 15 min, 2 × SSC for 5 min) and hybridized with the second probe. The blots were exposed for 40 min to Hyperfilm ECL (Amersham Biosciences).

DNA sequencing and sequence analysis

The *repAM*, *traJ* and 16S rRNA gene PCR products were cloned into the pGEM-T easy vector and either sequenced

commercially (GATC Biotech, Konstanz, Germany) or at our in-house sequence facility. The 16S rRNA gene PCR products were purified using a PCR purification kit (Qiagen) and sequenced with primers B8F, 338F (5′-ACTCCTACGGGRSGC-3′), 515R (5′-ACCGCGGCTGCTG GCAC-3′) and U1406R. Chromatograms were analyzed using CHROMAS 2.23 software and aligned with Clone manager 6.0. Phylogenetic trees were reconstructed by neighbour-joining with MEGA 3.1 (Kumar *et al.*, 2004) using CLUSTALW alignment and by calculating evolutionary distances by the Kimura-2 parameter method. Bootstrap values were calculated from 1000 replicate trees. Sequences were compared against the NCBI database.

Nucleotide sequence accession numbers

Sequences were deposited in the GenBank Nucleotide database with the following accession numbers: 16S rRNA gene sequences (EF196812–EF196832); *repAM* sequences (EF196833–EF196840); and *traJ* sequences (EF196841–EF196865).

Results and Discussion

Prevalence of pMEA *repAM* and *traJ* genes in *Amycolatopsis* strains

A total of 111 *Amycolatopsis* strains, originating from different geographical locations, were screened for the presence of pMEA *repAM* and *traJ* genes. Such pMEA-related *repAM* sequences were found in 10 of the 111 strains and pMEA-related *traJ* sequences in 26 of 111 strains (Table 1), indicating that pMEA-sequences, though not abundant, are widely distributed amongst members of the genus *Amycolatopsis*. The *A. methanolica, A. mediterranei* and *A. sacchari* strains, and four of the *Amycolatopsis* isolates (strains GY027, GY122, GY139, GY258), contained both *repAM* and *traJ* sequences. Plasmid isolation showed that strain *Amycolatopsis* GY027 contained several free replicating plasmids. Restriction analysis of the plasmid fraction, and subsequent hybridization with *repAM* and *traJ* probes, showed that the *repAM* and *traJ* sequences were present on a 16-kb plasmid (data not shown). Free replicating plasmids were not obtained from the other strains containing *repAM* and *traJ* sequences, indicating that elements carrying these sequences were most likely integrated in the chromosome.

Table 1. The number of *repAM* and *traJ* sequences found in 111 *Amycolatopsis* strains isolated from different geographical locations, as assessed by a combination of hybridization and PCR-based analysis

	No. of strains	*repAM*	*traJ*
European			
Type strains	1	1	1
Isolates	34	3	15
Australasian			
Type strains	9	3	3
Isolates	61	3	7
Other			
Type strains	6	0	0
Isolates	0	0	0
Total	111	10	26

Two isolates, GY034 and GY248, and *Sac. erythraea* only had a *repAM* sequence and 19 strains only a *traJ* sequence, results which suggest that pMEA *repAM* and *traJ* are not necessarily linked. It cannot be excluded that these isolates carry less conserved homologues of *repAM* or *traJ* that were missed in the screening procedure, but it appears more likely that *traJ* is associated with a different type of replication initiation protein and *vice versa*, emphasizing the mosaic structure of integrative elements (Osborn *et al.*, 2000) such as pMEA300 and pMEA100.

The pMEA-like elements appear to be globally distributed as *repAM* and *traJ* sequences were found in *Amycolatopsis* strains isolated from both Europe and Australasian countries. So far, *repAM* and *traJ* sequences have not been detected in the few *Amycolatopsis* strains isolated from other locations, notably Egypt (Henssen *et al.*, 1987) and the USA (Stapley *et al.*, 1972; Lechevalier *et al.*, 1986; Mertz & Yao, 1993; Labeda, 1995; Lee & Hah, 2001). Clearly, more strains from these regions need to be isolated and screened to enable firm conclusions to be drawn about the presence or absence of pMEA-like elements.

Amongst the 111 *Amycolatopsis* strains, *traJ* sequences were found to be more abundant than *repAM*, the latter forming the backbone of pMEA-like elements together with *xis*, *int* and *attP*. This suggests that these *traJ* sequences either have become integrated in the genome in the absence of *repAM* or have become associated with a different replication initiator gene. Alternatively, *traJ* may have spread by lateral gene transfer.

Geographic distribution of pMEA-sequences

Phylogenetic analysis of the deduced partial RepAM protein sequences revealed strong clustering with the RepAM protein sequences of either pMEA300 of *A. methanolica* or pMEA100 of *A. mediterranei*. The RepAM sequences from isolates GY027, GY034, and GY248 and from the *A. sacchari* and *Sac. erythraea* strains cluster with RepAM of pMEA300 (Fig. 2). On the other hand, the RepAM sequences of isolates GY122, GY139 and GY258 are closely related to RepAM of pMEA100 (∼95% identity) and to each other (∼99% identity).

Interestingly, analysis of the RepAM sequences also revealed a clear separation into two different geographic populations. Sequences that cluster with pMEA300 originated from strains isolated from Australasian countries,

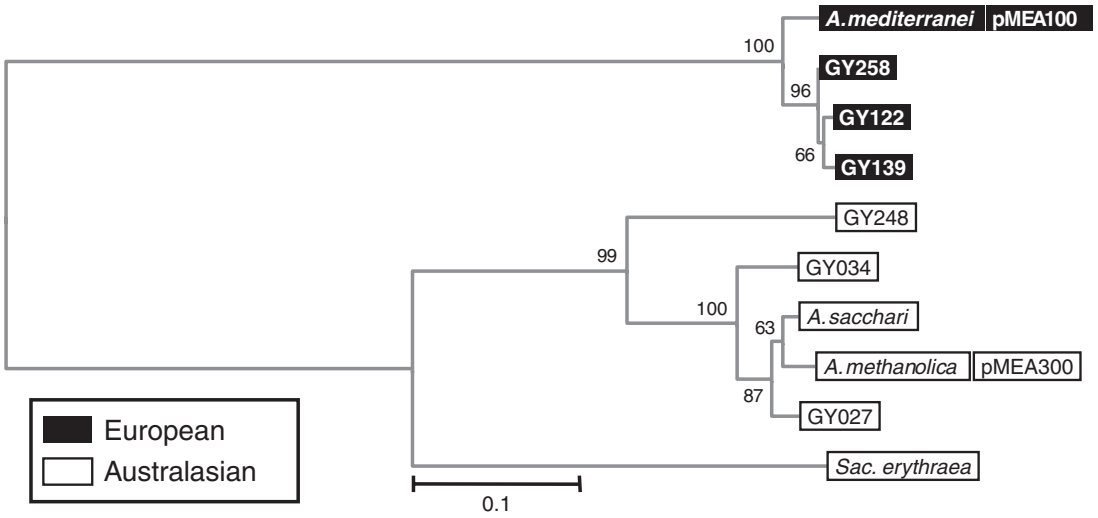

Fig. 2. Neighbour-joining tree of aligned deduced RepAM sequences from *Amycolatopsis* isolates, *Amycolatopsis* type strains and the type strain of *Saccharopolyspora erythraea*. Bootstrap values (1000 replicates) over 50% are shown. The scale bar represents 0.1 substitutions per nucleotide position.

whereas pMEA100-related RepAM sequences were found in strains isolated from European regions. The *A. methanolica* type strain carrying pMEA300 was isolated over 30 years ago in Papua New Guinea (Kato *et al.*, 1975; de Boer *et al.*, 1990). The *Sac. erythraea* strain was isolated from Philippine soil over 50 years ago (Carreras *et al.*, 2002). *Amycolatopsis* strains GY027, GY034 and GY248 originate from different locations in Australia (Tan *et al.*, 2006), whereas the *A. sacchari* strain was isolated a few years ago from the floor dust of a hemp factory in India (Goodfellow *et al.*, 2001). The *A. mediterranei* strain carrying pMEA100 was isolated from a pine arboretum on the South coast of France in 1957 (Margalith & Beretta, 1960) and the RepAM sequences that cluster together with $RepAM_{pMEA100}$ originate from strains isolated recently from the UK (Tan *et al.*, 2006). Despite several decades elapsing between the isolation of the type strains and these novel isolates, high sequence conservation can be observed between the sequences from these 'old' type strains and the fresh isolates, from similar geographic locations.

A similar clustering pattern and geographic distribution was observed for the TraJ protein sequences (Fig. 3). Most of the TraJ sequences derived from strains isolated from Australasian regions cluster with $TraJ_{pMEA300}$, whereas the majority of the TraJ sequences from European isolates cluster with $TraJ_{pMEA100}$. *Amycolatopsis* GY027 and *A. sacchari*, which have a pMEA300-related RepAM, also carry a TraJ that is most closely related to $TraJ_{pMEA300}$. Similarly, the TraJ sequences of *Amycolatopsis* isolates GY122, GY139 and GY258, which have a RepAM closely related to pMEA100, are closely related to $TraJ_{pMEA100}$. However, a small number of TraJ sequences did not follow this geographic distinction and formed a separate cluster containing both European and Australasian sequences. Interestingly, none of these strains contained a pMEA-like *repAM* sequence. Apparently, this separate class of TraJ proteins followed a distinct evolutionary route. The strong linkage of RepAM and the associated TraJ sequences suggest that they evolved in an integrated form. The loss of linkage to the European or Australasian cluster of the mixed TraJ cluster may have resulted from an early branching of the TraJ common ancestor before $TraJ_{pMEA100}$ and $TraJ_{pMEA300}$ became linked to RepAM and subsequent dispersal via horizontal gene transfer. The RepAM and the TraJ sequences of strains GY122, GY139 and GY258 that were isolated from the same location (Leazes Park) are highly conserved, which might suggest that they are isolates of the same strain. However, differences in their 16S rRNA gene sequences clearly show that this is not the case. A similar observation was made for the highly conserved Rep and Mob protein sequences on the pTA-like plasmids of *Bacillus* strains that were isolated from diverse geographical locations (Mason *et al.*, 2002).

Evolution of pMEA-like elements

The phylogenetic trees of RepAM and TraJ sequences indicated that there are two distinct populations of pMEA-like elements: one found in Europe and the other in Australia and Asia. The phylogenetic tree of 16S rRNA gene sequences of the *Amycolatopsis* isolates and type strains showed a similar distribution (Fig. 4). Apparently, *Amycolatopsis* dispersed at some point in its evolution, giving rise to the Australasian and European lineages. The linkage between the distribution of RepAM and TraJ with the

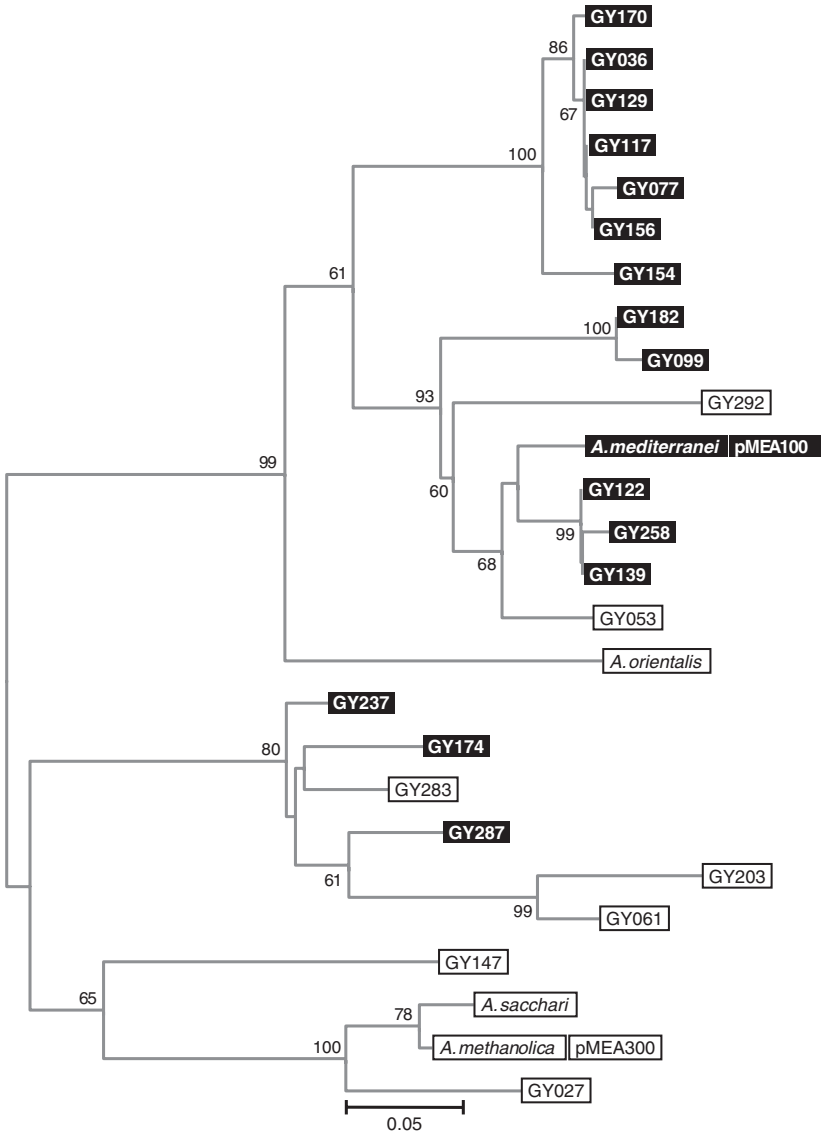

Fig. 3. Neighbour-joining tree of aligned deduced TraJ sequences from *Amycolatopsis* isolates and *Amycolatopsis* type strains. Bootstrap values (1000 replicates) over 50% are shown. The scale bar represents 0.05 substitutions per nucleotide position. Designation of geographical origin of the strains is as shown in Fig. 2.

chromosomal identifier, the 16S rRNA gene (Fig. 5), indicates that the pMEA-like elements coevolved with their hosts, suggesting that these elements evolved in their integrated form rather than by horizontal gene transfer of the free replicating form. In the latter case one would expect a much weaker linkage between a chromosomal identifier gene and the unique pMEA sequences. Close linkage between the element and host sequences was confirmed by the observation that the two isolated integrative elements pMEA100 and pMEA300 can only be transferred into their own (pMEA-free) host strains and cannot be exchanged (H. Kloosterman and E.M. te Poele, unpublished results).

Phylogenetic analysis of the RepAM and TraJ protein sequences shows that the genetic distance between the TraJ sequences is much smaller than that between the RepAM sequences (Figs 2 and 3). Possible explanations for this are that the amplified *traJ* region encodes a highly conserved domain of TraJ, or that the complete TraJ is more conserved than RepAM. The *repAM* fragment used in this study may encode a more variable region of RepAM. A similar higher diversity in Rep sequences was observed in the study of the pTA-type replication and mobilization genes of *Bacillus* plasmids (Mason *et al.*, 2002).

In conclusion, this study shows that pMEA-sequences are more widely distributed among *Amycolatopsis*. Linkage

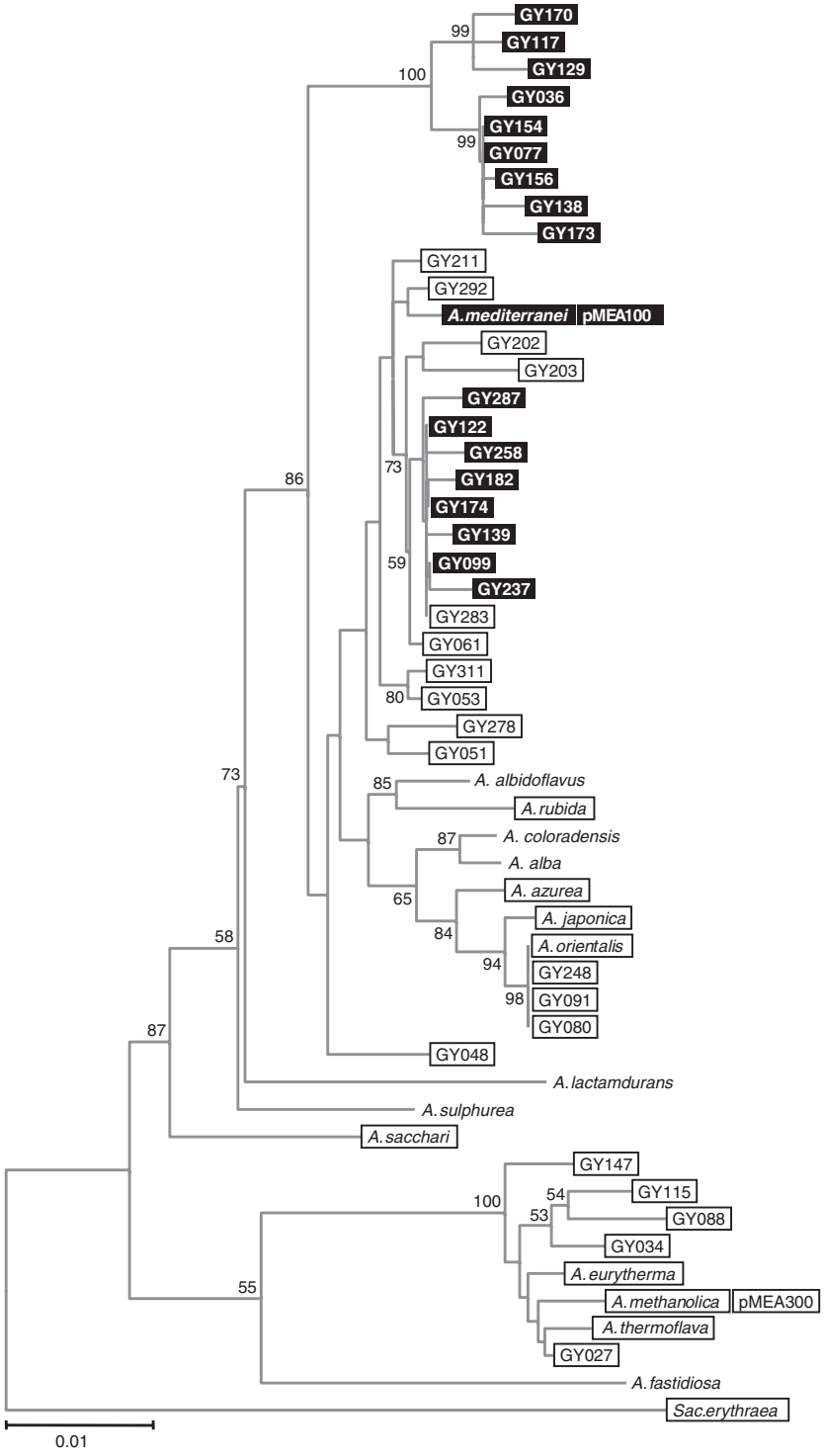

Fig. 4. Neighbour-joining tree of aligned 16S rRNA gene sequences (~1250 bp) from *Amycolatopsis* isolates and *Amycolatopsis* type strains. The type strain of *Saccharopolyspora erythraea* was used as an out-group. Bootstrap values (1000 replicates) over 50% are shown. The scale bar represents 0.01 substitutions per nucleotide position. Designation of geographical origin of the strains is as shown in Fig. 2.

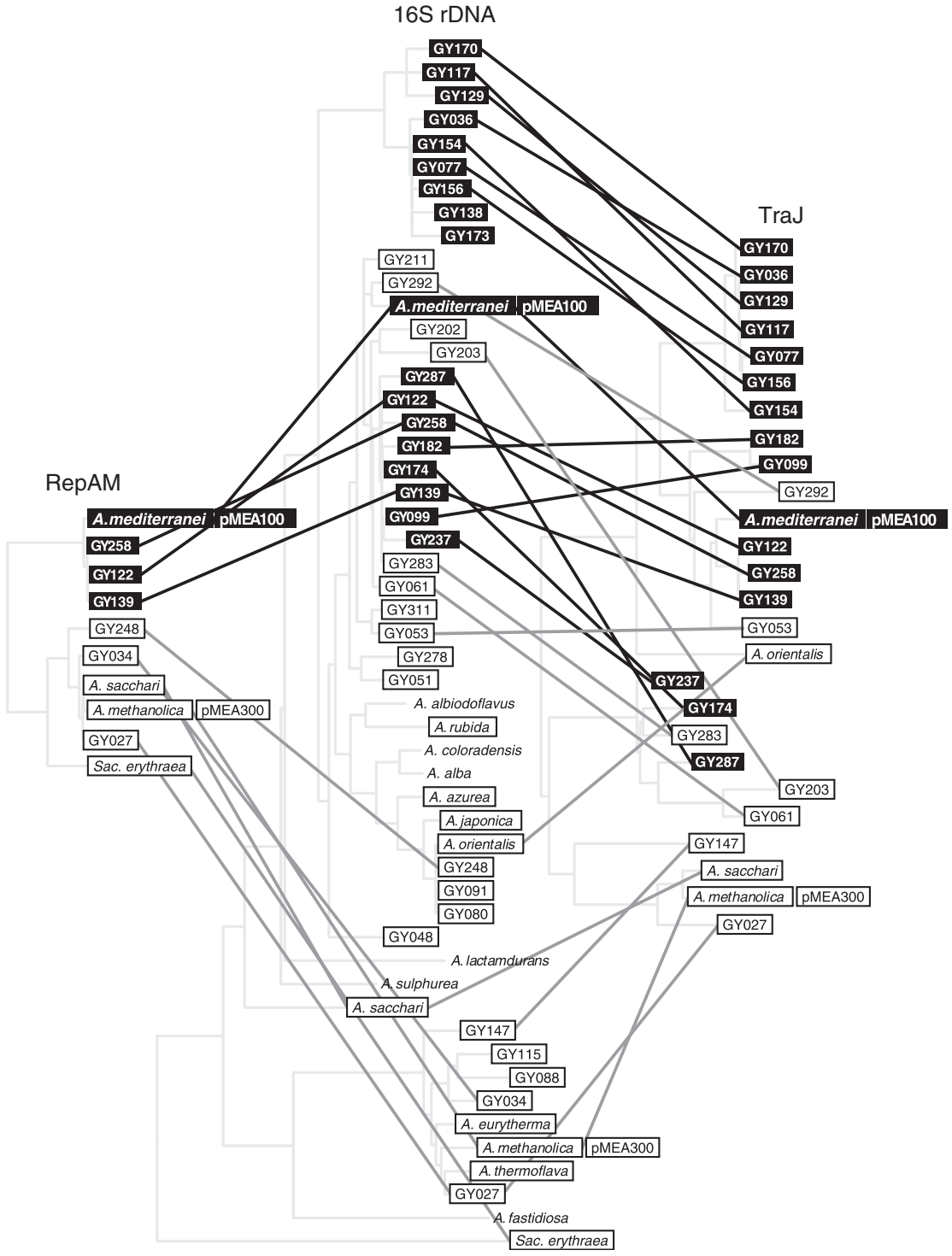

Fig. 5. Comparison of the phylogenetic relationship, as shown by connecting lines, of the *Amycolatopsis* 16S rRNA gene sequences with that of the RepAM and TraJ sequences reveals coevolution of the pMEA-like elements with their *Amycolatopsis* host strains. Designation of geographical origin of the strains is as shown in Fig. 2.

between the *repAM* and *traJ* genes and the 16S rRNA gene amongst the investigated strains suggests that pMEA-like elements mainly coevolved with their host in an integrated form. Isolation and analysis of *Amycolatopsis* strains from other parts of the world may reveal whether the pMEA elements consist of two distinct groups or whether additional geographic groups exist.

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