Identification of large linear plasmids in Arthrobacter spp. encoding the degradation of quinaldine to anthranilate

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Arthrobacter nitroguajacolicus Rü61a, which utilizes quinaldine as sole source of carbon and energy, was shown to contain a conjugal linear plasmid of approximately 110 kb, named pAL1. It exhibits similarities with other linear plasmids from Actinomycetales in that it has proteins covalently attached to its 5′ ends. Southern hybridization with probes for the genes encoding quinaldine 4-oxidase and N-acetylanthranilate amidase indicated that pAL1 contains the gene cluster encoding the degradation of quinaldine to anthranilate. A mutant of strain Rü61a that had lost pAL1 indeed could not convert quinaldine, but was still able to grow on anthranilate. Conjugal transfer of pAL1 to the plasmid-less mutant of strain Rü61a and to Arthrobacter nicotinovorans DSM 420 (pAO1) occurred at frequencies of $5 \cdot 10^{-4}$ and $2 \cdot 10^{-4}$ per recipient, respectively, and conferred the ability to utilize quinaldine. Five other quinaldine-degrading Gram-positive strains were isolated from soil samples; 16S rDNA sequence analysis suggested the closest relationship to different Arthrobacter species. Except for strain K2-29, all isolates contained a pAL1-like linear plasmid carrying genes encoding quinaldine conversion. A 478 bp fragment that on pAL1 represents an intergenic region showed 100 % sequence identity in all isolates harbouring a pAL1-like plasmid, suggesting horizontal dissemination of the linear plasmid among the genus Arthrobacter.

INTRODUCTION

Bacteria of the genus Arthrobacter are considered to be ubiquitous in soil and have been found to be among the predominant members of culturable communities from several terrestrial subsurface environments (Crocker et al, 2000). Among the explanations advanced for their ubiquity or even predominance in soil are their resistance to desiccation and nutrient depletion, and their nutritional versatility. Arthrobacter spp. utilize a wide and varied range of natural as well as xenobiotic compounds and thus may play a significant role in the mineralization of organic matter in the environment (Cacciari & Lippi, 1987).

Arthrobacter nitroguajacolicus strain Rü61a (formerly assigned to the species Arthrobacter ilicis) utilizes quinaldine (2-methylquinoline), a constituent of coal tar, as sole source of carbon and energy (Hund et al, 1990). Degradation via the anthranilate pathway (Fig. 1) is initiated by the oxidation of quinaldine to 1H-4-oxoquinidine, catalysed by quinaldine 4-oxidase (Qox). 1H-4-oxoquinoline 3-monoxygenase subsequently generates 1H-3-hydroxy-4-oxoquinidine, which undergoes 2,4-dioxygenolytic ring cleavage to form carbon monoxide and N-acetylanthranilate. This unusual mode of ring cleavage is catalysed by a cofactor-less 2,4-dioxygenase that does not share any similarity with aromatic ring cleavage dioxygenases, but seems to belong to the x/β-hydrolase fold superfamily of proteins (Fetzner, 2002). In the next step, an amidase (Amq) catalyses the hydrolysis of N-acetylanthranilate to anthranilate. We have characterized the gene cluster encoding this ‘upper part’ of the degradation pathway (Parschat et al, 2003); however, the genes of anthranilate utilization by strain Rü61a have not been identified so far.
Catabolic plasmids are not essential for viability, but may confer a selective advantage as they extend the metabolic versatility of their host. Several catabolic plasmids have been identified in Arthrobacter spp. The complete sequence of the 165 kbp plasmid pAO1, which enables Arthrobacter nicotinovorans to grow on L-nicotine (Brandisch & Decker, 1984), was determined recently (Igloi & Brandsch, 2003). Other plasmids of Arthrobacter spp. are involved in the degradation of 4-chlorobenzoate (Zaitsev et al., 1984), was determined recently (Igloi & Brandsch, 2003). All these catabolic plasmids of Arthrobacter spp. are circular or linear plasmids which encode catabolic traits (Dabrock et al., 1997; Kosono et al., 1997). Five other quinaldine-degrading strains isolated from soil were found to belong to the genus Arthrobacter, and four of them contain a very similar, if not identical, plasmid.

**METHODS**

**Bacterial strains, plasmids and growth conditions.** Arthrobacter sp. Rü61a was isolated from waste water (Dembek et al., 1989; Hund et al., 1990). Previous analysis of the variable region of its 16S rDNA had suggested that strain Rü61a belongs to the species *A. ilicus*; however, sequencing of the 16S rRNA gene performed in this work suggests its assignment to the species *A. nitroguajacolicus* rather than *A. ilicus*. Further quinaldine-degrading strains were isolated from soil samples by enrichment on mineral salts medium (Stephan et al., 1996) containing 2 mM quinaldine as sole source of carbon and energy, and supplemented with 1 ml vitamin solution l⁻¹ (Röger et al., 1990). An addition of cycloheximide (75 μg ml⁻¹) in the first enrichment steps prevented fungal contamination. The soil samples were taken from a field near Orte (Italy) (strain OF11), from a railway embankment (Oldenburg, Germany) (strain B2-6), from garden compost (Ocholt, Germany) (strain K2-29) and from a sewage site (Oldenburg, Germany) (strains KA).

**Arthrobacter** strains were grown in mineral salts medium (Stephan et al., 1996) with 1 g (NH₄)₂SO₄ l⁻¹ and vitamin solution (1 ml l⁻¹) (Röger et al., 1990), containing 4 or 2 mM quinaldine or 2 mM L-nicotinamide as carbon sources, or in nutrient broth yeast extract (NBYE) medium (Gartemann & Eichenlaub, 2001) or in Lysogeny Broth (LB) (Sambrook et al., 1989; Bertani, 2004) at 30 °C. Gram staining of the isolates was performed with the Gram staining kit from Fluka.

*A. nitroguajacolicus* Rü61a pAL1⁻ (RifR StrR) was obtained by repeated transfer of *A. nitroguajacolicus* Rü61a (RifR StrR) in LB and selection for loss of quinaldine utilization. Elimination of pAL1 from the genome was confirmed by PFGE and hybridization with pAL1-specific probes.

Plasmid pKGT452C (Gartemann & Eichenlaub, 2001, kindly supplied by R. Eichenlaub, was propagated in the methylase-negative strain Escherichia coli ET12567 (MacNeil et al., 1992) grown at 37 °C in LB containing ampicillin (100 μg ml⁻¹) and chloramphenicol (50 μg ml⁻¹). Arthrobacter strains carrying the transposon Tn1499Cβ...
were grown in the presence of 10 μg chloramphenicol ml⁻¹. *E. coli* DH5α (Woodcock et al., 1989), used for cloning of an intergenic fragment of pAL1 and related plasmids, was grown in LB containing 100 μg ampicillin ml⁻¹ when harbouring pUC18 derivatives.

**DNA techniques.** Genomic DNA of *Arthrobacter* strains was isolated according to the method of Rainey et al. (1996). Plasmid DNA from *E. coli* strains was isolated with the Qiagen Plasmid Mini kit. DNA restriction and agarose gel electrophoresis were carried out using standard procedures (Sambrook et al., 1989).

The 16S rRNA gene was amplified from total DNA using the oligonucleotide primers GM3F and GM4R (Muyzer et al., 1995). A 478 bp intergenic region that is located downstream of ORF5 in pAL1 (Parschat et al., 2003) was amplified using the primers frag-up (5'-AAAGTATCCAGCTCAGTGG-3') and frag-down (5'-ATCA-GCAGTGGCTACCAAAGTTC-3'), and total DNA of quinaldine-degrading strains as templates. Fragments were ligated into pUC18 (Vieira & Messing, 1982) and cloned in *E. coli* DH5α. PCR products were purified with the High Pure PCR Purification Kit (Roche Applied Science). DNA sequencing was performed with a 4000L DNA sequencer (LI-COR Inc., Biotechnology Division) using the dideoxy chain-termination method (Sanger et al., 1977). The CycleReader Auto Sequencing Kit (Fermentas) was used as specified by the manufacturer, together with oligonucleotide primers labelled with the synthetic fluorescent dye IRD800. For both the 16S rRNA genes and the intergenic regions, sequencing of both strands was done at least in duplicate.

Chloramphenicol resistant (CmR) transposon mutants of a spontaneous Ri² StrR mutant of *A. nitroguajacolicus* Ru61a pAL1 - and of *A. nicotinovorans* DSM 420 were obtained by electroporation of competent cells with the plasmid pKT452CfR isolated from *E. coli* ET12567 as described by Gartemann & Eichenlaub (2001). The presence of the cmx gene encoding a chloramphenicol exporter protein in the genome of these mutants was verified by PCR using the primer cmx-up (5'-CGGGGGATTGCTCCCCGCGATC-3') and cmx-down (5'-GTTY-GGAGGCCCGGAGCCACCAAG-3'), and genomic DNA as template.

The 16S rDNA sequences were aligned with published sequences from representative *Arthrobacter* species from the National Center for Biotechnology Information (NCBI) database. A phylogenetic tree of the quinaldine-degrading strains, different *Arthrobacter* spp. and the type strain of *Kocuria rosea* (DSM 20447) was constructed based on a sequence consensus length of 1427 nt of 16S rDNA (excluding gaps and Ns from the analysis). The fastDNAml program (Olsen et al., 1994) within the PAUP* 4.0 software (Swofford, 2002), which uses a maximum-likelihood approach, was used for the phylogenetic analysis. Binary alignments of the 16S rDNA sequences were performed with the program BestFit (Smith & Waterman, 1981).

**DNA probes and Southern hybridization.** DNA separated in agarose gels by PFGE was transferred by vacuum-blotting to nylon membranes (Porablot NY Plus; Machery-Nagel). Specific probes for the plasmid-localized genes *amq* (encoding *Amq*) and *qoxl* (encoding the large subunit of Qox) corresponded to nt 13941–14651 and 17490–21400, respectively, of the sequence deposited under EMBL accession no. CA537472. The probes were labelled with a digoxigenin derivative, using the DIG-High Prime DNA Labelling Kit (Roche Applied Science). Prehybridization, hybridization and colorimetric detection were performed following The DIG System User's Guide for Filter Hybridization (Boehringer Mannheim, 1995).

**Preparation of cell plugs, PFGE and isolation of linear plasmid DNA.** Cell plugs for PFGE were prepared by modifying the method described by Schenk et al. (1998). Cells of *Arthrobacter* spp. were grown either in mineral salts medium containing quinaldine or in LB to an OD₆₀₀ of 2–2.5. The washed bacterial cell pellets were preincubated in PIV buffer (1 M NaCl, 1 mM Tris/HCl, pH 7-6) containing 1 mg lysozyme ml⁻¹ (37 °C, 15 min). Cells were embedded in low-melting-point agarose, lysed using the method of Schenk et al. (1998) and equilibrated in TE buffer (10 mM Tris/ HCl, 1 mM EDTA, pH 8). Subsequent proteinase K treatment of the agarose plugs (if appropriate) was performed as described by Schenk et al. (1998); however, the samples were incubated for 5 h at 50 °C instead of overnight. PFGE was carried out in a contour-clamped homogeneous electric field apparatus (CHEF-DR III; Bio-Rad) using broad range agarose (1%, w/v, gels). Electrophoresis was performed at 5 V cm⁻¹ and 14 °C. The pulse time increased from 10 to 50 s, or from 40 to 90 s, during a 16 or 17 h run, respectively. Concatemers of λ DNA were used as size standard (Lambda DNA Ladder in InCert Agarose Gel Plugs; CAMBREX UK). To isolate pAL1, the relevant DNA band was excised from pulsed field (PF) agarose gels and the DNA was obtained by electroelution followed by precipitation with ethanol.

**S1 nuclease and exonuclease treatment of pAL1.** For treatment with S1 nuclease, agarose plugs with lysed cells of *A. nitroguajacolicus* Ru61a, or cell plugs after lysis and proteinase K treatment, were washed twice in S1 nuclease buffer (40–5 mM potassium acetate, 338 mM NaCl, 1·3 mM ZnSO₄, 6–8% w/v, glycerol, pH 7·0) and incubated with 20 or 50 Units S1 nuclease ml⁻¹ for 45 min at 37 °C. The reaction was stopped by adding 10 mM EDTA and cooling to 4 °C. Control experiments were performed without S1 nuclease. Plugs with lysed cells of strain Ru61a, treated with S1 nuclease, were washed in TE buffer and subsequently incubated for 2 h with 5, 10 or 20 Units λ exonuclease to examine if S1 nuclease treatment leads to the formation of unprotected 5′ ends.

To assess the sensitivity of proteinase-K-treated pAL1 DNA to exonucleases, plasmid DNA isolated from a preparative PF gel was embedded in an equal volume of 1% (w/v) low-melting-point agarose to prepare plugs containing approximately 100 ng plasmid DNA, and treated as described by Kalkus et al. (1993); however, 50 Units exonuclease III and 5 Units λ exonuclease were used. Remaining single-stranded plasmid DNA was removed by adding 0·6 Units mung bean nuclease ml⁻¹ and incubation at 37 °C for 30 min. For termination of the reaction, the buffer was replaced by TE buffer.

**Analysis of the degradative potential of *A. nitroguajacolicus* Ru61a pAL1.** To determine the ability of *A. nitroguajacolicus* Ru61a pAL1 to use quinaldine or intermediates of the anthranilate pathway as carbon and energy source, the carbon sources were added to the medium in concentrations of 0·1% (w/v) for 1H-4-oxoquinoline, *N*-acetylanthranilate and anthranilate, and 0·05% (v/v) for quinaldine. Mineral salts medium was inoculated with 5% (v/v) of an overnight culture in LB. Degradation of the substrates was monitored spectrophotometrically in the culture supernatant. Spectra were compared with those of authentic references diluted in the same medium.

**Mating experiments.** Filter mating was performed using *A. nitroguajacolicus* Ru61a as the donor, and the transposon mutants *A. nitroguajacolicus* Ru61a pAL1 - RifR StrR CmR and *A. nicotinovorans* DSM 420 CmR as recipients. Filters containing a mixture of donor and recipient cells at a 1:1 ratio were incubated for 24 h at 30 °C on the surface of an LB agar plate. Then the cells were suspended in 1 ml saline (0·9% NaCl) and appropriate portions were spread onto agar plates of mineral salts medium with 2 mM quinaldine as sole source of carbon and energy, and 10 μg chloramphenicol ml⁻¹.
was shown by amplifying part of the 6hlno gene (EMBL accession no. AJ507836) using the primer 6hlno-up (5'-GGCATTTTCTATTCCTGCTCTA-3') and 6hlno-down (5'-GCTCTCCAAGTTCCTTATGC- ACTC-3'). The gene encoding 6-hydroxy-L-nicotine oxidase (6HLNO) is part of the gene cluster involved in nicotine degradation encoded by pAO1 (Igloi & Brandsch, 2003).

RESULTS AND DISCUSSION

Identification of pAL1 from A. nitroguajacolicus Rü61a as a linear plasmid

PFGE of lysed cells of A. nitroguajacolicus Rü61a when treated with proteinase K revealed a discrete DNA band that was estimated to be 110 kb by comparison with linear standards (Fig. 2a, lane 3). Additional incubation with S1 nuclease, which can be used to linearize supercoiled circular plasmids (Barton et al., 1995), failed to change the mobility of the DNA molecule in the PF gel (Fig. 2a, lane 4). This behaviour is indicative of linear plasmids. The relative migration of proteinase-K-treated pAL1, determined after using two different pulse times, corresponded to linear concatemers of λ DNA under both running conditions (not shown), confirming that the DNA molecule is linear.

The sum of the sizes of all fragments obtained by restriction digests with various restriction endonucleases was 90–110 kb, which roughly corresponds to the size estimated for the undigested molecule. The linear plasmid was designated pAL1 (for first linear Arthrobacter plasmid).

Proteins are attached to the 5' ends of pAL1

In contrast to the native plasmid from cells lysed without any proteinase or nuclease treatment (Fig. 2a, lane 1), pAL1 molecules migrated into the PF gel after treatment of the cell plugs with proteinase K (Fig. 2a, lane 3) or SDS (not shown), suggesting that proteins are attached to the DNA. The terminal proteins of linear replicons of streptomycetes and other actinomycetes were previously proposed to be covalently bound to the 5' ends (Kalkus et al., 1993; Ravel et al., 1998; Polo et al., 1998; Yang et al., 2002; Stecker et al., 2003). As a consequence, the linear plasmid is insensitive to 5'–3' exonuclease, but sensitive to 3'–5' exonuclease.

pAL1 was isolated from a preparative PF gel and tested for sensitivity to E. coli exonuclease III and phage λ exonuclease, which hydrolyse dsDNA in the 3'–5' and 5'–3' directions, respectively. As shown in Fig. 3, DNA exonuclease III

![Fig. 2. PFGE separation of total DNA of A. nitroguajacolicus Rü61a (a) and Southern hybridization with an amq-specific probe (b). Prot. K, treatment of samples with (+) or without (−) proteinase K; S1 nuc., treatment with (+) or without (−) S1 nuclease. M, λ DNA concatemers as size standard. PFGE programme: increase of pulse duration from 10 to 50 s during a 16 h run.

![Fig. 3. Sensitivity of pAL1 to exonuclease treatment. PFGE of pAL1 isolated by electrolefution from a preparative PF gel of proteinase-K-treated total DNA of A. nitroguajacolicus Rü61a. Lanes: 1, λ DNA concatemers as size standard; 2, pAL1 as isolated; 3–7, pAL1 treated with exonuclease III (lane 3), exonuclease III and mung bean nuclease (lane 4), 5'-exonuclease (lane 5), 3'-exonuclease and mung bean nuclease (lane 6), and mung bean nuclease (lane 7). PFGE programme: increase of pulse duration from 10 to 50 s during a 16 h run.](image-url)
completely degraded pAL1, whereas φ exonuclease did not, indicating that the 5′ end of pAL1 is protected, even after proteinase K treatment. The functionality of the φ exonuclease was proven by digestion of pUC18 plasmid DNA linearized with Smal (not shown). The result is consistent with the proposal of a covalent bond between the 5′ end and the terminal protein which is not hydrolysed by proteinase K. For Streptomyces linear replicons, attachment of the 5′ phosphate to the hydroxyl group of a serine residue on the terminal protein has been suggested (Yang et al., 2002).

Replication of Streptomyces linear plasmids and chromosomes has been shown to proceed bi-directionally from (an) internal origin(s) towards the telomeres, generating replicative intermediates that contain 3′-strand overhangs (Musialowski et al., 1994; Chang & Cohen, 1994; Chang et al., 1996; Redenbach et al., 1999; Qin et al., 2003). To produce full-length duplex DNA molecules, the recessed 5′ ends of the lagging strands have to be extended (‘patched’). The terminal proteins of Streptomyces linear replicons were proposed to have a priming role for telomere patching (Yang et al., 2002; Bao & Cohen, 2001, 2003). An analogous function is assumed for the terminal proteins of the linear plasmids from other actinomycetes; however, their exact role in replication has not been investigated yet.

Quite surprisingly, treatment of lysed cells of strain Ru61a with S1 nuclease seemed to be sufficient for pAL1 to enter the PF gel (Fig. 2a, lane 2). Sequential treatment of embedded, lysed cells with S1 nuclease and φ exonuclease indicated that the terminal proteins of pAL1 are not removed by S1 nuclease treatment. However, when plugs containing lysed cells after equilibration in TE buffer were incubated in S1 nuclease buffer without addition of the enzyme, pAL1 showed the same mobility in the PF gel as after incubation with S1 nuclease or after treatment with SDS. In contrast, pAL1 did not migrate into the PF gel when either ZnSO4 or NaCl was omitted from the S1 nuclease buffer, or when the salt concentration in the buffer was decreased to 50 or 200 mM NaCl. We suggest that S1 nuclease buffer has a denaturing effect on the terminal proteins of pAL1. When the soluble protein fraction of crude extracts of A. nitroguajacolicus Ru61a was incubated in this buffer for 45 min at 4 and 37 °C, about 40 and 50 % of the total protein was precipitated, respectively, demonstrating the potential of the buffer as a denaturant for proteins.

pAL1 encodes quinaldine conversion to anthranilate

We previously showed that a 10·8 kb HindIII fragment of DNA from A. nitroguajacolicus Ru61a contains the genes encoding the enzymes of the ‘upper part’ of the anthranilate pathway of quinaldine degradation, i.e. the conversion of quinaldine to anthranilate (Fig. 1) (Parschat et al., 2003). Southern blotting following PFGE and hybridization with a probe specific for amq (Fig. 2b) and with a qoxL-specific probe showed that this 10·8 kb fragment is actually part of pAL1. A mutant that spontaneously had lost the ability to utilize quinaldine, 1H-4-oxoquinaldine and N-acetylanthranilate was analysed by PFGE and Southern hybridization. pAL1 indeed was not detected in the mutant and the genomic DNA did not show hybridization signals with probes specific for the qoxL and amq genes (Fig. 4), confirming that the enzymes involved in anthranilate formation from quinaldine are encoded by the plasmid and were lost in the mutant. However, A. nitroguajacolicus Ru61a pAL1− was able to grow on anthranilate, indicating that a pathway for anthranilate utilization is chromosomally encoded. The anthranilate metabolism of strain Ru61a was previously proposed to proceed via catechol and the ortho cleavage pathway (Hund et al., 1990).

pAL1 is transmissible by conjugation

To obtain a selectable marker for the recipient strains of conjugation experiments, CmR mutants of A. nitroguajacolicus Ru61a pAL1− (RifR StrR) and A. nicotinovorans DSM 420 were generated by transposon mutagenesis. These strains, which are not able to utilize quinaldine, were used for mating with A. nitroguajacolicus Ru61a. About 1 week after plating the mating mixture on mineral salts medium containing quinaldine as sole carbon source and chloramphenicol, CmR A. nitroguajacolicus and A. nicotinovorans transconjugants were obtained at frequencies of 5·4 × 10−4.
and $2.0 \times 10^{-4}$ colonies, respectively, per recipient. From each mating experiment, four transconjugants were analysed by PCR experiments. Both the cmx gene and the pAL1-located amq gene were identified in all transconjugants examined. To exclude the possibility that a pAO1 derivative carrying the cmx gene had been transferred to A. nitroguajacolicus Rü61a, the identity of transconjugants that resulted from the mating of A. nicotinovorans DSM 420, CmT with A. nitroguajacolicus Rü61a was assessed by 16S rDNA analysis. The results confirmed that interspecies transfer of pAL1 from A. nitroguajacolicus to A. nicotinovorans had occurred. The ability of the A. nicotinovorans transconjugants to grow on L-nicotine as sole carbon source and the detection of the pAO1-specific 6hho gene suggested the presence of the circular plasmid pAO1, beside pAL1.

Most of the linear plasmids identified in Actinomycetes were reported to be transmissible plasmids (Meinhardt et al., 1997; Picardeau & Vincent, 1998; Ravel et al., 2000). Plasmid-mediated conjugation probably enables these soil bacteria to share advantageous genetic elements. Linear conjugative replicons such as pAL1, pBD2 of R. erythropolis BD2 encoding isopropylbenzene catabolism (Dabrock et al., 1994; Stecker et al., 2003), the linear plasmids of rhodococci involved in polychlorinated biphenyl degradation (Shimizu et al., 2001; Kosono et al., 1997) or those of alkene-assimilating Mycobacterium strains (Coleman & Spain, 2003) may play an important role in the dissemination of catabolic capabilities and may be important for the evolution of degradation pathways.

The quinaldine-degrading strains belong to the genus Arthrobacter, and four out of five new isolates contain a pAL1-like plasmid

All quinaldine-degrading strains that were isolated from the soil samples were Gram-positive and 16S rDNA analysis suggested that they all belong to the genus Arthrobacter. Based on a sequence of 1432 nt, the highest similarity of the 16S rDNA of strain Rü61a was found with the 16S rRNA gene from A. nitroguajacolicus DSM 15232 (EMBL accession no. AJ512504) (99.9 % identity in the binary comparison), whereas binary alignments with 16S rDNA of A. ilicis DSM 20138 (EMBL accession no. AIRNA16S) and A. aurescens DSM 20116 (EMBL accession no. AARNNA16S) revealed identities of 99-0 and 99.6 %, respectively. We thus suggest that strain Rü61a belongs to the species A. nitroguajacolicus rather than A. ilicis. Remarkably, the 16S rDNA sequences of strain OF11 and the acyl-homoserine lactone-utilizing strain Arthrobacter sp. VAI-A (Flagan et al., 2003) showed 99.9 % identity (comparison of 1431 nt). Fig. 5 shows the phylogenetic positions of the isolates compared to related Arthrobacter spp. and K. rosea.

Separation of genomic DNA from lysed cells by PFGE indicated the presence of DNA showing the same mobility as pAL1 in strains B2-6, KA1-1, KA4-2 and OF11 when the samples had been treated with proteinase K. Each of these DNA molecules hybridized with probes specific for amq (Fig. 6) and qoxL, indicating that in all these strains, quinaldine utilization proceeds via the anthranilate pathway. Several Arthrobacter strains appear to contain additional extrachromosomal DNA besides the pAL1-like plasmid (Fig. 6a). The quinaldine-degrading isolate K2-29 contains extrachromosomal DNA which in PFGE migrates faster than pAL1 (Fig. 6a, lane 6); this DNA element did not hybridize with pAL1-specific probes, suggesting that genes encoding quinaldine conversion are localized either on the chromosome or on a plasmid that is not separated by the PFGE protocol. However, S1 nuclease treatment of genomic DNA from strain K2-29 to linearize circular plasmid DNA and subsequent PFGE did not reveal any additional DNA band on the gel.

Restriction fragment patterns of plasmid DNA were used to assess the interspecies variability of the pAL1-like plasmids. Although overlapping fragments not separated in the agarose gels complicated the analysis, the results suggested that
restriction patterns were conserved between the plasmids of strains Rü61a, B2-6, KA1-1, KA4-2 and OF11 (Fig. 7). To further address the presumed high similarity of the linear plasmids, a non-coding region that in pAL1 is located downstream of a putative transporter gene (ORF5) (Parschat et al., 2003) was amplified by PCR and sequenced. Using genomic DNA of strain KA2-29 as template, it was not possible to amplify this 478 bp fragment. The PCR amplicons obtained with template DNA of the plasmid-containing strains showed 100% sequence identity, suggesting horizontal dissemination of the linear catabolic plasmid pAL1 among the genus Arthrobacter. However, it is an intriguing but open question which contributions – besides quinaldine degradation – of the linear megaplasmid may provide the reason for its widespread occurrence among Arthrobacter spp.

Fig. 6. PFGE separation of total DNA of Arthrobacter spp. (a) and Southern hybridization with an amq-specific probe (b). Lanes: 1 and 9, λ DNA concatemers as size standard; 2, A. nitroguajacolicus Rü61a; 3, strain B2-6; 4, A. nicotinovorans DSM 420; 5, strain KA1-1; 6, strain K2-29; 7, strain OF11; 8, strain KA4-2. PFGE programme: increase of pulse duration from 10 to 50 s during a 16 h run.

Fig. 7. Restriction patterns of pAL1 and pAL1-like plasmids from different quinaldine-degrading Arthrobacter strains (0.7% agarose gel). Plasmid DNA isolated by electro-elution from a preparative PF gel from strains B2-6 (lanes 1), KA4-2 (lanes 2), A. nitroguajacolicus Rü61a (lanes 3), OF11 (lanes 4) and KA1-1 (lane 5) was digested with PstI (a), SacI (b), and Sacl and Safl (c). Lanes M, size marker containing DNA fragments of 10, 8, 6, 5, 4, 3, 2-5, 2 and 1-5 kb.
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