Volume 17 Number 18 1989 Nucleic Acids Research

Similarity of minus origins of replication and flanking open reading frames of plasmids pUB110, pTB913 and pMV158

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Received June 13, 1989; Revised and Accepted August 21, 1989 EMBL accession nos X15669, X15670

ABSTRACT

Plasmids pMV158 and pTB913, originating from *Streptococcus agalactiae* and a thermophilic *Bacillus* respectively, were sequenced to completion. Both contained a BA3-type minus origin of replication and an RS_A-site, believed to constitute a site-specific recombination site. These two regions were more than 99% homologous to the corresponding regions of the *Staphylococcus aureus* plasmid pUB110. Deleting the BA3-type minus origin resulted in the accumulation of a considerable amount of single-stranded DNA, both in *L.lactis* subsp. *lactis* and *B.subtilis*, indicating that this minus origin was functional in both bacterial species. Like pUB110, both plasmids contained an open reading frame encoding a putative plasmid recombination enzyme (Pre protein), which was located downstream of the RS_A-site. On the basis of sequence comparisons between pUB110, pMV158, pTB913, pT181, pE194, pNE131 and pT48 two distinct families of RS_A-sites and Pre proteins could be distinguished.

INTRODUCTION

Plasmid pMV158 is a 5.4 kilobasepair (kb) multicopy plasmid originally isolated from *S.agalactiae* (1). It expresses tetracycline resistance in both Gram-positive and Gram-negative hosts. pLS1 was derived from pMV158 by deletion of a 1.1 kb *Eco* RI-fragment. The sequence of this plasmid was completely determined (2).

The replication functions of pLS1 consist of an origin, a 5.1 kilodalton (kD) trans-acting repressor, RepA, and the replication initiator protein, RepB (3). Replication of pLS1 has been shown to occur by an asymmetric rolling-circle mechanism (3,4) similar to that of small multicopy staphylococcal plasmids like pUB110 (5) and pC194 (6), and the *Escherichia coli* phages M13 and ΦX174 (7,8).

Most small Gram-positive plasmids replicate via the rolling-circle mode of replication (9). Their minus origins, which are the initiation sites for the conversion of single- to double-stranded plasmid replication intermediates can be divided into three groups (9), namely: (a) palA, present on, among others, pC194 (10), pE194 (11), pT181 (12) and pLS1 (4); (b) BA3, present on pUB110 (13,14,15); and (c) 'stab', present on pTA1060 (16), pLS11 (17) and pBAA1 (18).

pLS1 contains a single-stranded DNA conversion signal of the palA-type. This minus origin was efficiently recognized in *Streptococcus pneumoniae*, but not in *E.coli* or *B.subtilis* (4). We observed that the conversion of single-stranded pMV158 DNA in *L.lactis* was far more efficient than that of pLS1, suggesting the presence of an alternative conversion signal on the 1.1 kb *Eco*R I-fragment lacking in pLS1. This prompted us to determine the sequence of this fragment. Part of the sequence had a high degree of homology with pUB110, a 4548 basepairs (bp) multicopy plasmid, derived from *S.aureus*, encoding

Table 1. Bacterial strains and plasmids

bacterial strain or plasmid	properties	source or reference
Bacteria		
B.subtilis 8G5	trpC2, tyr1, met, his, nic, purA, ura, rib	(26)
L.lactis subsp. *lactis* IL1403		(45)
Plasmids		
pMV158	Tc^r, 5.4 kb	(1)
pLS1	Tc^r, 4.3 kb derivative of pMV158	(2)
pUB110	Km^r, $bleo^r$, 4.5 kb	(19)
pUB3	Km^r, $bleo^r$, 4.2 kb derivative of pUB110 lacking the minus origin	This work
pTB913	Km^r, $bleo^r$, 4.5 kb	(22)
pTB3	Km^r, $bleo^r$, 4.2 kb derivative of pTB913 lacking the minus origin	This work

resistances to both kanamycin (Km) and bleomycin (bleo) (19). The nucleotide sequence of this plasmid was described (20).

pTB19 is a 27 kb low copy plasmid isolated from a thermophilic *Bacillus* species. It confers tetracycline- and kanamycin resistance (21) and contains two replication functions, RepA and RepB (22). During selection on kanamycin a specific deletion derivative, pTB913 (4.5 kb), was obtained from pTB19 (22). The sequence of the RepB region and kanamycin resistance gene was recently determined (23,24). Comparison with pUB110 showed a high

Fig. 1. Nucleotide sequence of the 1,139 bp *Eco* RI-fragment of pMV158. The numbering of this sequence is consistent with the numbering of pLS1 (2). The positions of the BA3-type minus origin, RS_A site, RBS and the N-terminal part of the Pre protein are indicated. The C-terminal part of the Pre protein is described as ORF D in (2).

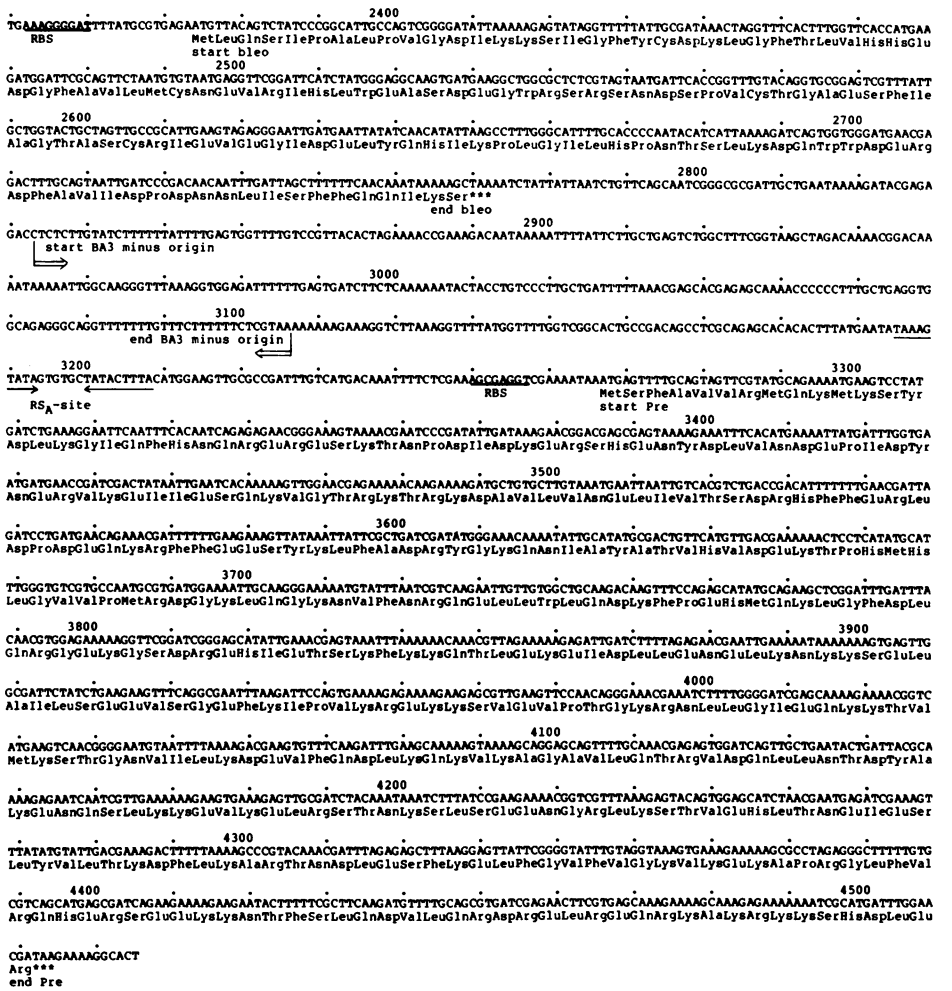

Fig. 2. Nucleotide sequence of part of pTB913 (positions 2350 to 4525). The first nucleotide of pTB913 as sequenced by Muller et al. (24) was chosen to represent position 1 in the numbering of pTB913.
The positions of the bleomycin resistance gene, the BA3-type minus origin, the RS_A site, RBS and the Pre protein are indicated.

degree of homology between the replication initiation proteins. Likewise the kanamycin resistance genes were also very identical (20). Because of these similarities it is conceivable that pTB913 would also replicate according to the rolling-circle model. This prompted us to investigate the extent of homology between pTB913 and pUB110 by sequencing pTB913 to completeness.

In this paper we show that plasmids pMV158 and pTB913 contain a conversion signal (nearly) identical to the BA3-type minus origin of pUB110. Furthermore we show the presence of a nucleotide sequence, similar to that of a recently described site-specific

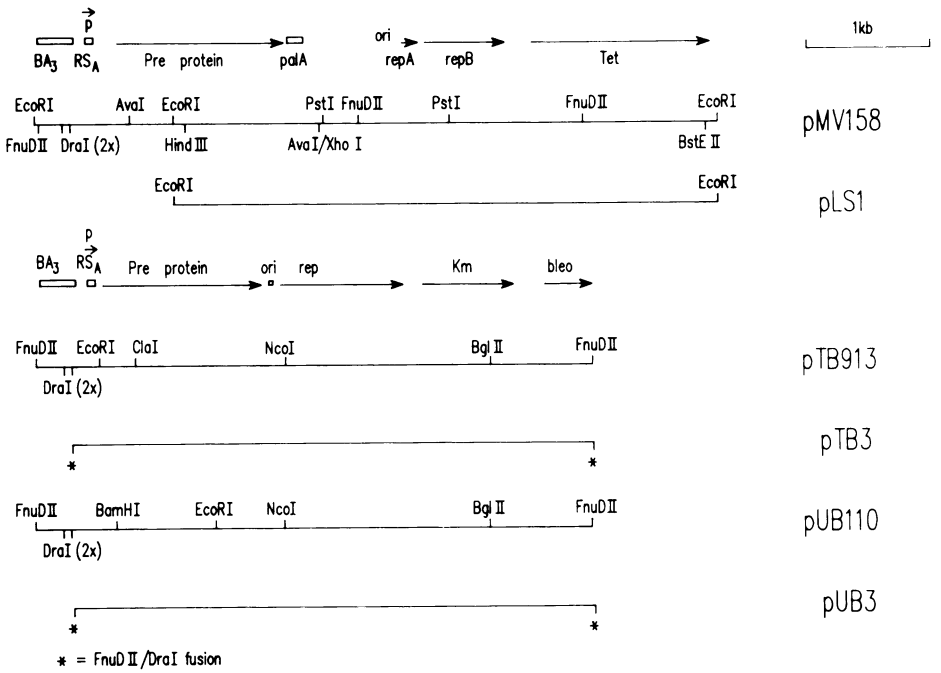

Fig. 3. Restriction maps of pMV158, pLS1, pUB110, pUB3, pTB913 and pTB3. The positions of the relevant regions are indicated.

recombination function for the *S.aureus* plasmids pT181 and pE194 (25). This function consists of a 24 nucleotide palindromic structure, designated as the RS_A-site, and an open reading frame (ORF) encoding a Pre protein (plasmid recombination enzyme) of approximately 49 kD. It has been assumed that this protein promotes Rec-independent plasmid cointegrate and multimer formation at the RS_A-site in *S.aureus* (25).

MATERIALS AND METHODS

Bacterial strains, plasmids and media

The strains and plasmids used are listed in Table 1. TY broth (27) was used for culturing *E.coli* and *B.subtilis*. For plating, TY broth was solidified with 1.5% agar. *L.lactis* was cultured and plated on M17 (28) broth and agar supplemented with 0.5% glucose (GM17). Kanamycin and phleomycin were used at final concentrations of 10 μg/ml and 2 μg/ml, respectively, for *B.subtilis*. Tetracycline was added at a final concentration of 4 μg/ml for both *L.lactis* and *B.subtilis*, and at a final concentration of 10 μg/ml for *E.coli*.

Isolation of plasmid and total DNA

Plasmid DNA was isolated from *B.subtilis* and *E.coli* by the method of Ish-Horowicz and Burke (29) and, with minor modifications of the same procedure, from *L.lactis* (30). Whole cell lysates were prepared and isolated as described before (13).

Restriction enzyme reactions, molecular cloning and gel electrophoresis

Restriction enzymes and endonuclease S1 were purchased from Boehringer GmbH (Mannheim, Federal Republic of Germany) and used as recommended by the supplier.

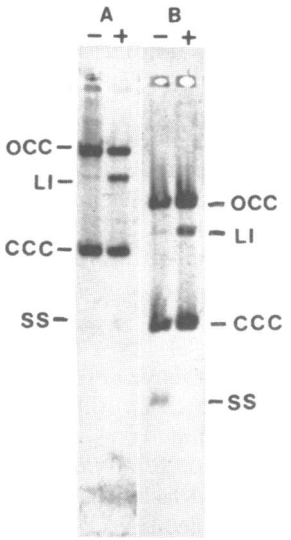

Fig. 4. Whole cell lysates of *B.subtilis* 8G5 harbouring pMV158 or pLS1 without (−) or with (+) treatment with endonuclease S1.
A: *B.subtilis* 8G5 (pMV158)
B: *B.subtilis* 8G5 (pLS1)
The position of single stranded DNA (ss), covalently closed circle DNA (ccc), linear DNA (li) and open circle DNA (oc) are indicated.

Molecular cloning was performed as described by Maniatis et al. (31). Digested DNA was analyzed in 0.8% horizontal agarose gels in TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2mM EDTA, 0.5 μg of ethidiumbromide per ml, pH 8.0). DNA fragments were isolated from gels using the freeze-squeeze method (32).

DNA sequencing

The nucleotide sequences of pMV158 and pTB913 were determined to completion by sequencing double-stranded plasmid DNA in both orientations, using the T7 DNA polymerase sequencing kit (Pharmacia, Uppsala, Sweden), which is based on the dideoxy chain termination method (33). Synthetic oligonucleotides were kindly provided by Unilever Research Laboratories (Vlaardingen, The Netherlands). In the computer-assisted sequence comparisons MicroGenie software (Beckman, Palo Alto, Calif.) was used.

Transformation of L.lactis subsp. lactis, B.subtilis and E.coli

L.lactis was transformed by electroporation as described before (34) with minor modifications. The cells were washed twice with 12.5 ml of ice-cold double distilled water, once with 12.5 ml of 50 mM EDTA (pH 7.0), and finally with 12.5 ml of 0.3 M sucrose. The cell pellets were resuspended in 0.4 ml of 0.3 M sucrose. The DNA solutions, usually 20 μl, containing 5 μg of DNA, and 0.4 ml of cell suspensions, were mixed and electroporation was carried out at 12500 V/cm in a pre-cooled cuvette (0°C). The capacitance was set at 25 μF and the Pulse Controller™ (Bio Rad) at 200 Ω. After the electric pulse, the cell suspensions were immediately transferred to 10 ml GM17-sucrose (GSM17) medium (35) and, after allowing time for expression (2 h at 30°C), cells were harvested, resuspended in 1 ml GSM17 medium and plated on GSM17 plates containing

selective antibiotics. Competent cells of *B.subtilis* were prepared and transformed as described by Bron et al. (13). *E.coli* was transformed according to Mandel and Higa (36).

Blot hybridizations

After electrophoresis in agarose gels (0.8%) the DNA was transferred to GeneScreen Plus filters by the protocol of Southern, as modified by Chomczynski and Qasba (37). Random primed DNA was labelled with digoxigenin-dUTP with the Nonradioactive DNA Labeling and Detection Kit, (Boehringer GmbH, Mannheim, Federal Republic of Germany), and subsequently denatured for 10 min at 100°C. The hybridization and staining steps were carried out according to the manufacturer's instructions.

RESULTS

Nucleotide sequence of pMV158

pLS1 is a broad host range deletion derivative of pMV158 that is able to replicate in both Gram-positive and Gram-negative bacteria (2). Although its minus origin of the palA-type appears to be functional in *S.pneumoniae* (4), no efficient conversion of single-stranded DNA was observed in either *L.lactis* or *B.subtilis*. This will be documented in one of the following paragraphs. Because pLS1 lacks a 1.1 kb *Eco* RI-fragment of pMV158, we assumed that an alternative single-stranded DNA conversion signal, which was functional in both *L.lactis* and *B.subtilis*, would be located on this part of pMV158. This prompted us to analyze the *Eco* RI-fragment in more detail. The sequence of the *Eco* RI-fragment is presented in Fig. 1. It is 1,139 bp in length which makes the total size of pMV158 5541 bp. Computer analysis showed a region in the *Eco* RI-fragment, extending from positions 3197 to 3615 in pMV158 (coordinates refer to pLS1 [2]), which exhibited more than 99% homology with the pUB110 fragment between positions 1134 and 1552 (coordinates according to reference 20). This region of pUB110 contains the minimal functions for the conversion of single- to double-stranded DNA (13,15) plus the RS_A-site (25,38). The homology between pUB110 and pMV158 ended 9 bp downstream of the RS_A-site. These results indicate that in addition to the palA-type conversion signal on pMV158 (4) a second conversion signal of the BA3-type is present on this plasmid.

At 112 bp downstream of the RS_A-site a potential ribosome binding site (RBS) was present, which was followed by an ORF of 1485 bp. This ORF extends into the part of pMV158 that had been sequenced before in pLS1 (2). The free energy (dG°) of binding between this RBS and the 3′ end of the 16S rRNA of *B. subtilis* was calculated to be −22 kcal/mole (39). Upstream of this putative RBS a possible promoter was present from positions 3576 to 3581 (−35 region) and positions 3598 to 3603 (−10 region). This would place the −10 region within the RS_A-site.

Nucleotide sequence of pTB913

pTB913 is a 4.5 kb deletion derivative of pTB19, a plasmid isolated from a thermophilic *Bacillus* (21,22). On the part of the plasmid of which the sequence was determined before (23,24) a kanamycin resistance gene and the repB gene are present, both showing more than 99% homology with the corresponding genes from pUB110 (20). The homology of the rep genes suggested that, like pUB110, pTB913 replicates via the rolling-circle mechanism and, therefore, that a minus origin was likely to be present on the part of pTB913 of which the sequence had not yet been determined. We sequenced this 2176 bp fragment to test this idea and to examine whether further homology existed between pUB110 and pTB913.

The sequence is presented in Fig. 2. By including the length of the newly sequenced

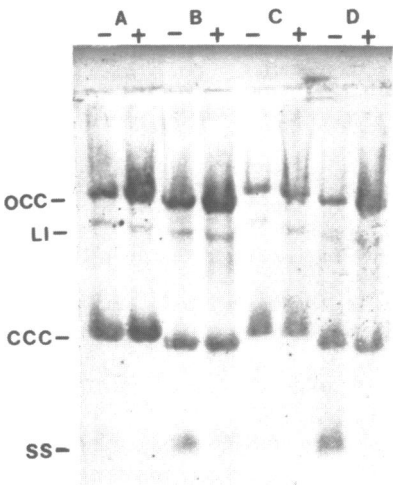

Fig. 5. Whole cell lysates of *B.subtilis* 8G5 harbouring pUB110, pUB3, pTB913 or pTB3 without (–) and with (+) treatment with endonuclease S1.
A: *B.subtilis* 8G5 (pUB110)
B: *B.subtilis* 8G5 (pUB3)
C: *B.subtilis* 8G5 (pTB913)
D: *B.subtilis* 8G5 (pTB3)
The positions of single stranded DNA (ss), covalently closed circle DNA (ccc), linear DNA (li) and open circle DNA (oc) are indicated.

part the total size of pTB913 was determined to be 4,525 bp. Computer analysis showed a region extending from positions 2350 to 3198, which was completely identical to the sequence of pUB110 between positions 2188 to 1340 (20). This region includes the bleomycin resistance gene and the BA3-type minus origin. Therefore, like pUB110 and pMV158, pTB913 contains the BA3-type minus origin. The bleomycin resistance genes of pUB110 and pTB913 conferred resistance to this antibiotic both to *B.subtilis* 8G5 and *B.stearothermophilus* CU21 (results not shown).

Compared to pUB110, the RS_A-site of pTB913 contained a one basepair substitution in the loop of the potential stem-loop structure (See fig 2). A potential RBS was present between position 3250 and 3257 followed by an ORF starting at position 3267. A potential promoter was present at positions 3178 to 3183 (–35 region) and positions 3200 to 3205 (–10 region). This would, like pMV158 and pUB110 place the putative –10 region within the RS_A-site. The free energy (dG°) of binding between the 3′ end of the 16S rRNA of *B.subtilis* and the RBS was calculated to be –24 kcal/mole (39). The ORF extended from positions 3267 to 4511.

Effect of the BA3 regions on the accumulation of single-stranded plasmid DNA in B.subtilis and L.lactis subsp. lactis

pLS1, which lacks the BA3-type minus origin (Fig. 3), was constructed by in vitro deleting the 1,139 bp *Eco* RI-fragment from pMV158 (40). pUB3 is a 4320 bp pUB110 derivative from which the BA3-type minus origin was deleted by removing the *Fnu* DII (position 1549)–*Dra* I (position 1321) fragment (Fig. 3) (20). This deletion should remove the entire functional minus origin (15). pTB3 is a 4298 bp pTB913 derivative from which the BA3-type

	IR	loop	IR	homology end points
	⟹		⟸	
pUB110	TAAAGTATA	GTGTGT	TATACTTTA	CTTGGAAGT
pMV158	TAAAGTATA	GTGTGT	TATACTTTA	CATGGAAGT
pTB913	TAAAGTATAG	TGTG	CTATACTTTA	CATGGAAGT
pT181	AAGTCTA	GTGTGT	TAGACTT	AAA CTAT
pE194	AAGTCTA	GTGTGT	TAGACTT	TA TGAAATCTAT
pNE131	AAGTCTA	GTGTGT	TAGACTT	TAATG
pT48	AAGTCTA	GTGTGTTT	TAGACTT	TAATG

Fig. 6. Comparison of the RS_A regions of plasmids pUB110 (20), pMV158, pTB913, pT181 (25), pE194 (25), pNE131 (41) and pT48 (42).
The positions of the inverted repeats (IR), loops and the homology end points are indicated. Underlinings represent differences among the nucleotide sequences.

minus origin was deleted by removing the *Fnu* DII – *Dra* I fragment corresponding to that described above for pUB110 (Fig. 3).

We examined whether the absence of the BA3-type minus origin affected the accumulation of single-stranded DNA from these deletion derivatives in *B. subtilis* 8G5 and *L. lactis* subsp. *lactis* IL1403. Whole cell lysates from cells harbouring pMV158, pTB913 or pUB110 or their corresponding deletion derivatives pUB3, pLS1 and pTB3 respectively were isolated. The lysates were devided in two portions, one of which was treated with endonuclease S1 to remove all single-stranded DNA present. After electrophoresis and Southern blotting the filters were hybridized to non-radioactive labeled plasmid DNA of the corresponding plasmids. From the results, shown in Figs. 4 and 5, it is clear that deletion of the BA3-type minus origin from all three plasmids resulted in the accumulation of considerable amounts of single-stranded plasmid DNA (about 30% in mass), both in *B. subtilis* and in *L. lactis*. This indicates that the BA3-type minus origin is active in both organisms.

Comparison of the RS_A-sites and Pre proteins

The sequence comparison of pMV158 and pTB913 with pUB110 revealed a high degree of homology, not only within the BA3-type minus origin, but also within the nearby RS_A site. Other plasmids have been described to contain an RS_A-like site and in Fig. 6 the RS_A regions of plasmids pUB110, pMV158, pTB913, pT181 (25), pE194 (25), pNE131 (41) and pT48 (42) are compared. This comparison shows that two related classes of RS_A-sites can be distinguished. In the RS_A sites of the first group, comprising pUB110, pMV158 and pTB913, the nucleotide sequence 5′ TAAAGTATA 3′ and its complement are present as an inverted repeat (IR). No further homology was observed between the various members of this group beyond 9 bp on the 3′ end of the IR. In the other group, comprising pT181, pE194, pNE131 and pT48 the IR was formed between the sequence 5′ AAGTCTA 3′ and its complement.

Adjacent to the RS_A sites of pUB110, pTB913, pMV158, pE194 and pT181 ORF's were present. In pE194 and pT181 it has been suggested that these ORF's encode site-specific recombination (Pre) proteins (25). This prompted us to examine whether the ORF's of pTB913 and pMV158 might encode similar proteins. An alignment of the first 210 amino acids of all five proteins is presented in Fig. 7. A very high degree of homology (85%) was observed between the N-terminal 200 amino acids of the proteins encoded by pUB110 and pTB913, suggesting a similar function for this part of the proteins. Although the level of homology observed between the N-terminal 200 amino acids of the proteins encoded by ORF-D of pMV158 and the Pre protein of pUB110 was substantially less (45%),

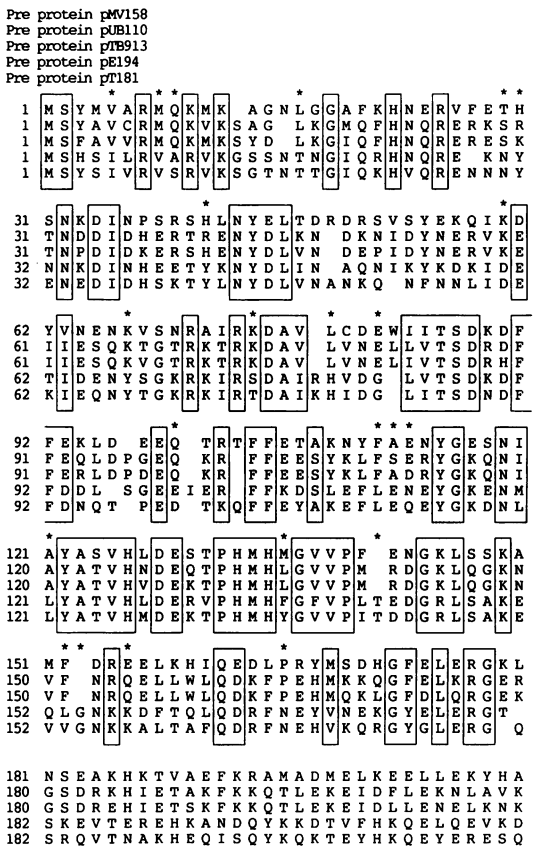

Fig. 7. Alignment of the first 210 amino acids of the putative Pre proteins of pMV158, pUB110 (20), pTB913, pT181 (25) and pE194 (25). Amino acids, conserved in all five proteins are boxed. Asterisks indicate amino acids differing between the two subgroups (pMV158, pUB110 and pTB913 versus pE194 and pT181), but conserved within those subgroups.

boxes of conserved amino acids were present. These boxes were present in all five proteins shown in Fig. 7. This suggests that at least the N-terminal parts of these proteins share similar functions. The results also show that the Pre proteins of pMV158, pUB110 and pTB913 on one hand were more related to each other than to those of pE194 and pT181 on the other (Fig. 7, indicated with asterisks). No conserved stretches of amino acids were observed in the C-terminal portions of the five proteins (results not shown).

DISCUSSION

pMV158, originally isolated from *S.agalactiae*, replicates according to the rolling-circle model (2,3,4). Although a minus origin of the palA-type (44) is present on the pLS1 deletion variant of pMV158 (4), the present work indicated that, in contrast to pMV158, less efficient conversion of single- to double-stranded DNA was observed with pLS1 in *B.subtilis*. Similar results were obtained in *L.lactis* (results not shown). This suggested that an alternative

minus origin might be present on the 1.1 kb *Eco* RI-fragment, which had been deleted from pMV158 to construct pLS1. This prompted us to sequence pMV158 to completion. The results (Figs. 1 and 4) show that, indeed, a minus origin is present, and is more than 99% homologous with the BA3 conversion signal of pUB110 (13,14, 15). This means that pMV158 contains two minus origins: one of the palA-type (9,44) and one of the BA3-type. To our knowledge pMV158 is the first naturally occurring plasmid which has been found to contain two minus origins. We showed that pLS1, lacking the BA3-type minus origin, gave less efficient conversion of single- to double-stranded DNA than pMV158 in both *B.subtilis* 8G5 and *L.lactis* IL1403 (Fig. 4). This indicates that the BA3-type minus origin is required for the efficient conversion to double stranded plasmid molecules, at least in these two organisms. Apparently, the palA-type minus origin is relatively inefficient in *B.subtilis* and *L.lactis*. The latter result is in agreement with the observation of del Solar et al. (4), who showed that the palA-type minus origin is not very efficient in *B.subtilis*. The observation that pMV158 contains two different minus origins may, at least in part, explain the broad host range character of this plasmid: the palA sequence was reported to be efficiently used in *S.pneumoniae* (4), and, somewhat less efficiently, in *E.coli* (4). The functioning of this pMV158 encoded palA-type minus origin in other streptococci (pMV158 was isolated from *S.agalactiae*) and in staphylococci, in which other members of the palA family are active (9,44), has so far not been examined. Interestingly, the BA3-type minus origin appeared to be highly efficient in two non-related Gram-positives: *B.subtilis* and *L.lactis*. This is a rather unusual finding, since the activity of most of the minus origins studied so far is limited to one or only a few closely related species (9).

pTB913 is a spontaneous deletion derivative of the thermophilic *Bacillus* plasmid pTB19. The high level of similarity (99%) of its replication functions with those of pUB110 (23,24) suggested that, like pUB110, pTB913 would replicate according to the rolling circle-model. Our search to demonstrate a cognate minus origin resulted in the detection of a conversion signal identical to the BA3-type minus origin of pUB110. Therefore, the present paper extends BA3-type minus origins to three plasmids, isolated from non-related species: pUB110 (from *Staphylococcus aureus*); pMV158 (from *Streptococcus agalactiae*) and pTB913/pTB19 (from a thermophilic *Bacillus*). This observation lends support to the view put forward by Gruss and Ehrlich (9), that the replication systems of many rolling-circle-type Gram-positive plasmids are closely related. Due to the high recombination potential of the single-stranded replication intermediates, large blocks of functionally related sequences could be shuffled around between plasmids. The BA3-type minus origin might be an example of such a block. Since in the plasmids studied sofar (pUB110, pMV158, pTB913) sequence divergence occurs just beyond the RS_A-site, we speculate that this potential stem-loop structure was, in an unknown way, involved in this process. Interestingly, the comparison between pMV158 and pUB110 (or pTB913) showed that the sequence divergence at the other end also occurred at a potential stem-loop structure (near the *Fnu* DII site at position 1547 of the pUB110 sequence). This stem-loop structure contains an inverted repeat of 10 bp ($dG° = -11.2$ kcal/mole) and a loop region of 3 bp.

On one side of the BA3-type minus origins of pMV158, pTB913 and pUB110 a small potential stem-loop structure was present, designated before as the RS_A site (25,38). Comparison of the RS_A sites of these plasmids with those of pT181, pE194, pNE131 and pT48 showed two distinct, but related groups of RS_A sites (Fig. 6). In the first group, comprising pTB913, pMV158 and pUB110, the homology end point was located at 9 bp

beyond the RS_A site. A similar observation was made in the second group, comprising pT181, pNE131, pT48 and pE194.

The RS_A sites of pTB913, pMV158, pUB110, pT181 and pE194 were followed by ORFs encoding proteins which showed considerable levels of similarity, exclusively in the N-terminal part of the proteins, suggesting that these proteins are related and have a common function. For pT181 and pE194 these proteins, designated as Pre proteins (plasmid recombination enzyme), have been shown to be involved in the Rec-independent formation of cointegrates between plasmids containing RS_A sites (25,38). It was suggested (25) that after binding to the RS_A site the Pre protein promotes the formation of cointegrates with other plasmids containing RS_A sites. All five proteins contain conserved positively charged amino acids (Arg and Lys). It is conceivable that these amino acids are involved in the binding of the Pre protein to the RS_A site (43). Interestingly, the Pre proteins of pTB913, pMV158 and pUB110 are more related to each other than to those of pT181 and pE194. These groupings coincide with the catagories of RS_A sites described above. This suggests that the various Pre proteins recognize specific RS_A sites.

It has been speculated that the Pre protein may be involved in plasmid maintenance (25). Although unequivocal proof is lacking, results from our laboratory (13) indicate that deletion of the pre gene of pUB110 derivatives, larger than 10 kb, results in increased rates of segregational plasmid loss. In addition, we have recently shown that a frameshift mutation in the gene encoding the Pre protein destroyed conjugative mobilization of pMV158 (46). Therefore, we suggest that the interaction of the RS_A site and Pre protein not only serves a function in plasmid maintenance, but also contributes to the distribution of small antibiotic resistance plasmids among Gram-positive bacteria. The latter feature might help in the spread of minus origins, such as BA3, and other plasmid functions among different Gram-positive bacterial backgrounds.

ACKNOWLEDGEMENTS

This work was supported by the Biotechnology Action Program (B.A.P.) of the Commision of the European Communities and the Programma Commissie Biotechnologie of the Ministry of Economic Affairs (The Netherlands).

We thank B. Kazemir and H. A. B. Wösten for partly sequencing pTB913 and pMV158, respectively, Dr. A. Ledeboer (Unilever Research Laboratories, Vlaardingen, The Netherlands) for kindly providing synthetic oligonucleotides, W. J. J. Meijer for fruitful discussions and H. Mulder for preparing the figures.

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