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repABC-Type Replicator Region of Megaplasmid pAtC58 in *Agrobacterium tumefaciens* C58

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Abstract The region responsible for replication of the megaplasmid pAtC58 in the nopaline-type *Agrobacterium tumefaciens* strain C58 was determined. A derivative of a ColE1 vector, pBluscript SK-, incapable of autonomous replication in *Agrobacterium* spp, was cloned with a 7.6-kb BglII-HindIII fragment from a cosmid clone of pAtC58, which contains a region adjacent to the operon for the utilization of deoxyfructosyl glutamine (DFG). The resulting plasmid conferred resistance to carbenicillin on the *A. tumefaciens* strain UIA5 that is a plasmid-free derivative of C58. The plasmid was stably maintained in the strain even after consecutive cultures for generations. Analysis of nested deletions of the 7.6-kb fragment showed that a 4.3-kb BglII-XhoI region sufficiently confers replication of the derivative of the ColE1 vector on UIA5. The region comprises three ORFs, which have high homologies with *repA*, *repB*, and *repC* of plasmids in virulent *Agrobacterium* spp. including pTiC58, pTiB6S3, pTi-SAKURA, and pRiA4b as well as those of symbiotic plasmids from *Rhizobium* spp. Phylogenic analysis showed that *rep* genes in pAtC58 are more closely related to those in pRiA4 than to pTi plasmids including pTiC58, suggesting that the two inborn plasmids, pTiC58 and pAtC58, harbored in C58 evolved from distinct origins.

Key words: *Agrobacterium tumefaciens*, *rep* genes, plasmid, pAtC58

The genus *Agrobacterium* comprises pathogenic soil bacteria, which cause crown gall tumor or hairy-root in susceptible plants. The plant pathogens generally harbor large (in general, 200–400 kb in size) unit-copy plasmids, called Ti (tumor-inducing) and Ri (root-inducing) plasmids [15, 33, 39, 40]. Functions associated with tumorigenicity, opine catabolism, replication, and conjugal transfer encoded by these plasmids have been intensively studied. In addition to Ti or Ri plasmids, numerous *Agrobacterium* strains also contain large, unit-copy megaplasmids [33]. These plasmids generally are not essential for the virulence of the pathogens [31]. Molecular and genetic studies of the biological functions encoded by these plasmids have not been well characterized, and compared to Ti or Ri plasmids.

The regions responsible for replication of some Ti and Ri plasmids have been defined. The replication regions of Ti or Ri plasmids in *Agrobacterium* spp. are distinct from those of other types of plasmids. The root hair-inducing plasmid pRiA4b from *A. rhizogenes* strain A4 encodes three ORFs responsible for replication [23]. In several types of Ti plasmids, such as the octopine/mannityl opine-type Ti plasmid pTiB6S3, the nopaline-type Ti plasmid pTiC58, and the nopaline-type pTi-SAKURA, highly conserved replication regions have been identified [20, 25, 36, 37]. All of these plasmids contain highly conserved three ORFs, commonly designated as *repA*, *repB*, and *repC*, constituting members of the *repABC*-type family. Whereas other types of plasmids often have genes encoding the functions of replication and partitioning located separately in distant loci, these genes encoded by the *rep*ABC-type plasmid family are closely linked and constitute an operon [11, 25]. RepA and RepB proteins are similar to ParA/SopA and ParB/SopB, respectively, which are proteins involved in partition of plasmid P1 and F [8]. These two proteins are thought to be responsible for plasmid stability and a copy number control. RepA also serves as a *trans*-acting incompatibility factor [28] and a negative regulator of the transcription of the *repABC* operon of pSym plasmid in *Rhizobium etli* and pTi plasmid in *Agrobacterium tumefaciens* [26, 29]. The function of RepC is essential for replication, and has been suggested to be a replication initiator protein [7, 24]. The replication region of the nopaline-

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type pTiC58 is mapped nearby *trb/traI* and its structure is similar to those of the octopine-type pTiB6S3 and pTi-SAKURA [20, 25, 36, 37].

In addition to pTiC58, nopaline-type strain C58 harbors a megaplasmid, pAtC58. This plasmid has been known to be self-conjugative [40] and to have a positive effect on *vir* gene induction [22]. Apparently, pTiC58 and pAtC58 in the strain are compatible with each other, indicating that they belong to distinct incompatibility groups. The nucleotide sequences and regulation of *rep* genes in pTiC58 have been studied [20, 25, 26], but elements for replication in pAtC58 have not yet been studied. In our previous study of pAtC58, a cosmid clone was isolated, which contains an essential region for utilization of the Amadori opine, deoxyfructosyl glutamine (DFG) [2, 3, 5, 18]. The DNA nucleotide sequence of a part of the region showed homology with that of *repC* from *A. rhizogenes* plasmid pRiA4b [2], leading us to hypothesize that this region is responsible for replication of the megaplasmid.

In this study, we defined the precise region responsible for replication in pAtC58 and determined its DNA nucleotide sequence. We identified three genes and showed that those genes are functional in the replication and maintenance of the plasmid. DNA nucleotide sequence analysis of those genes suggested that pAtC58 is phylogenically closer to Ri plasmids than to Ti plasmids.

MATERIALS AND METHODS

Bacterial Strains, Growth Conditions, and Reagents

Bacterial strains used in this study are listed in Table 1. Luria-Bertani (LB) broth (Gibco BRL) was used as a rich medium for *Escherichia coli* and nutrient broth (NB) (Difco Laboratories, Detroits, MI, U.S.A.) for *A. tumefaciens*. AB medium containing 0.5% glucose (ABG) [9] was used as a defined medium for *A. tumefaciens*. MG/L was used to prepare competent *A. tumefaciens* cells for electroporation and TYM for *E. coli* transformation [9, 32]. *A. tumefaciens* cells were grown at 28°C and *E. coli* at 37°C. Antibiotics were used at the following concentration: for *A. tumefaciens*, tetracycline (Tc) at 1.5 μg/ml, carbenicillin (Cb) at 50 μg/ml; for *E. coli*, tetracyclin (Tc) at 10 μg/ml, ampicillin (Ap) at 50 μg/ml.

DNA Manipulations and Transformation

Plasmids were isolated from *E. coli* and *A. tumefaciens* by alkaline lysis procedures [12, 32]. Restriction enzyme digestions and ligations of DNA were performed as recommended by the manufacturers (MBI Fermentas, New England Biolabs, Takara). Plasmids were introduced into *E. coli* as described by Sambrook and Russell [32], and into *A. tumefaciens* by electroporation [9] or by biparental mating using *E. coli* S17-1.

Table 1. Bacterial strains and plasmid used in this study.

Strains and plasmid	Relevant genotype, phenotype, or characteristics[a]	Reference or source
Escherichia coli		
DH5α	*supE44 Δ lacU169*(Φ80*lacZ Δ* M15)*hsdR17 recA1 endA1 gyrA96 thi-1 relA*	Our collection
S17-1	RP4-2-Tc::Mu-Km::Tn*7*, *pro*(r- m+) Mob^+ Tp^r	[34]
Agrobacterium tumefaciens		
UIA5	pAtC58 and pTiC58-cured derivative of the nopaline-type strain C58C1RS, Rif^r	Our collection
Plasmids		
pBluescript SK (-)	Cloning vector, ColE1derivative, Ap^r (confers Cb^r on *A. tumefaciens*)	Stratagene
pRK415	Broad-host-range IncP cloning vector, Tc^r	[16]
pCH-1	A 13.8-kb BglII/HindIII fragment from pAtC58 cloned into pRK415	[2]
pBSAtO	A 7.6-kb BglII-HindIII fragment from pCH1 cloned into pBluescript SK, Cb^r	This study
pBS-X2	A 4.3-kb BglII-XhoI fragment from pBSAtO cloned into pBluescript SK	This study
pBS-At#[b]	A series of nested deleltion clones, derived from pBSAtO (Fig. 1)	This study
pBS-AtApa4	A derivative of pBSAtO with a deletion of a 2.5-kb HindIII-ApaI fragment	This study
pBS-At(-)Sa5	A derivative of pBSAtO containing a SalI-HindIII 4.9-kb fragment	This study
pBS-Rep	A derivative of pBS-X2 with a deletion of the region downstream to *repC*	This study
pBS-ΔpRep	A derivative of pBS-X2 with a deletion of the region upstream to *repA*	This study
pBS-ΔA	A derivative of pBS-X2 with a deletion of *repA*	This study
pBS-ΔB	A derivative of pBS-X2 with a deletion of *repB*	This study
pBS-ΔBC	A derivative of pBS-X2 with a deletion of the region downstream to *repA*	This study
pBS-Δ*igs*	A derivative of pBS-X2 with a deletion of the region between *repB* and *C*	This study
pBS-ΔABi	A derivative of pBS-X2 with a deletion of *repAB* and *igs*	This study
pBS-ΔC	A derivative of pBS-X2 with a deletion of the region downstream to *repB*	This study

[a]Abbreviations: Ap^r, ampicillin resistance; Cb^r, carbenicillin resistance; Rif^r, rifampicin resistance; Sm^r, streptomycin resistance; Tc^r, tetracycline resistance; Tp^r, trimethoprim resistance.

[b]Physical map of the deletions is diagrammatically shown in Fig. 2.

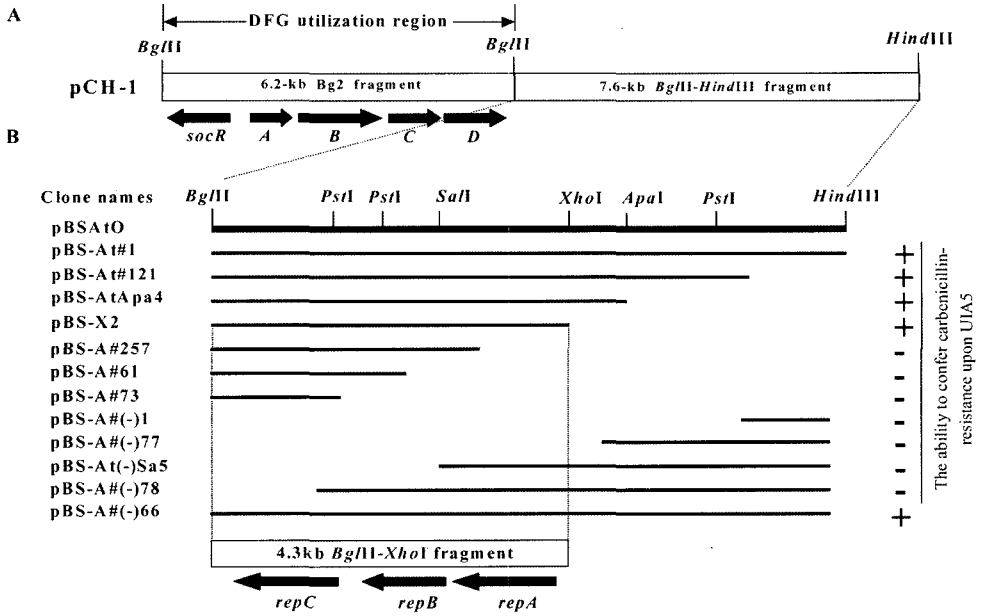

Fig. 1. Genetic map of subclones from pBSAtO and the ability of each subclone to allow the replication of pBluescript derivatives in UIA5.

A. Genetic map of pCH-1 [2]. B. Genetic map of subclones of the 7.6-kb BglII-HindIII fragment. The replication of each clone was indirectly tested by examining the ability to confer carbenicillin-resistance upon UIA5, a plasmid-free derivative of C58 and examining the presence of the plasmids by DNA preparations. A 4.3-kb BglII-XhoI fragment was the smallest subclone to allow pBluescript to replicate in UIA5. C. Organization of *rep* genes.

Construction of Nested Deletions of the *rep* Region and Test of Their Ability to Confer Replication

Nested deletions of pBSAtO were made by the method described previously [32]. Each deletion was verified by DNA nucleotide sequencing. The physical map of the deletions is described in Fig. 1. Resulting deletions were transformed into UIA5 and tested for the ability to grow on ABG solid medium supplemented with 50 μg/ml carbenicillin.

Determination and Analysis of DNA Nucleotide Sequences

DNA nucleotide sequence was determined on both strands using an ABI-373A automated DNA sequencer (Perkin Elmer Corp., Foster City, CA, U.S.A.). The search for homologous sequences was performed using the BLAST programs [1] at the National Center for Biotechnology Information (Bethesda, MD, U.S.A.). Complete nucleotide sequences of *rep* genes are deposited in the GenBank database under the accession number of AF283811.

Test for Stability of pBluescript Derivatives Containing Various *rep* Regions in UIA5

We tested the ability of various parts of *rep* regions cloned in pBluescript SK(−) to allow the replication and maintenance of the plasmid in UIA5 by examining if each clone confers the resistance to carbenicillin, a derivative of β-lactam antibiotic, on the cell. After five generations of consecutive cultures without antibiotic selection, each clone was serially diluted and spread on NB agar plate without antibiotics. Among those, 100 colonies were randomly chosen and patched onto plates with or without carbenicillin. For those clones resistant to the antibiotics, the presence of plasmids was confirmed by plasmid mini-preparation [12]. The efficacy of the stability was expressed by the ratio between numbers of cells harboring the plasmid (and hence resistant to carbenicillin) and those sensitive to carbenicillin.

Test for Induction of *rep* Expression by *Agrobacterium* Autoinducer (AAI) Molecules

To test whether the expression of *rep* genes in pAtC58 was induced by quorum sensing, β-galactosidase activities from cells of UIA5, harboring various *lacZ*-fusions on pCH-1 generated by Tn*3*HoHoI, were measured quantitatively [4] in the presence and absence of synthetic *N*-3-oxooctanoyl-L-homoserine lactone molecule, which is an AAI molecule in the C58 strain [41]. The AAI molecule, *N*-3-oxooctanoyl-L-homoserine lactone, was chemically synthesized and purified as described previously [17, 41].

Results and Discussions

Isolation and Characterization of the Replication Region of pAtC58

A subclone of pAtC58, pCH-1, contains the operon for DFG catabolism [2]. A partial DNA nucleotide sequence of the region upstream to the operon showed homology with *repC* of pTiC58, which is responsible for replication

Table 2. Relatedness among the putative gene products of pAtC58 *rep* genes and those of *repABC*-type genes from representative strains belonging to Rhizobiaceae.

	Relatedness (%similarity/%identity) to *rep* gene products of:								
	A. tumefaciens				*A. rhizogenes*	*R.* sp NGR234	*R. leguminosarum*	*R. etli*	
Genes of pAtC58	pTiC58 (AF060155)[a]	C58 Linear Chromosome (AE007870)	pTiSAKURA (AB016260)	pTiB6S3 (M24529)	pRiA4b (X04833)	pNGR234 (AE000068)	pRL8JI (X89447)	p42a (AF528525)	p42d (U80928)
repA	71/57	70/49	71/57	72/56	68/47	69/47	70/52	71/53	65/47
repB	55/40	50/32	55/40	55/40	51/32	52/33	50/33	55/37	53/34
repC	65/47	60/43	65/47	65/41	81/68	72/57	71/58	76/60	80/65

[a]Accession numbers in the GenBank database.

of the plasmid [2]. This finding led us to assume that the 7.6-kb BglII-HindIII region (Fig. 1A) may encode genes responsible for replication of the plasmid. To verify this hypothesis, we tested the ability of the 7.6-kb BglII-HindIII region from pCH-1 to confer the replication of a plasmid on *A. tumefaciens*. Thus, the 7.6-kb fragment was subcloned into pBluescript-SK(−), a derivative of the ColE1 plasmid that cannot autonomously replicate in *Agrobacterium*. The resulting construction was named pBSAtO. The plasmid was transformed into UIA5, a plasmid-free derivative of C58. The transformants were patched onto NB solid medium, and each colony was tested for the resistance to carbenicillin at 50 μg/ml in ABG solid medium. From the carbenicillin-resistant colonies, plasmid DNAs were purified and restriction profiles were examined. The profiles were identical to that of the original pBSAtO plasmid DNA (data not shown). From these cells, the plasmid was isolated and was introduced into fresh UIA5 cells. The cells were again resistant to carbenicillin and harbored the pBSAtO plasmid. These results showed that the 7.6-kb region encodes functions allowing the ColE1 plasmid to replicate in UIA5.

To define a precise region in the 7.6-kb fragment required for a stable replication of a plasmid, we constructed several subclones and nested deletions of pBSAtO. Detailed descriptions of these subclones are given in Table 1, and a genetic map of those constructions is shown in Fig. 1B. The clone pBS-X2, which carries the 4.3-kb BglII-XhoI fragment, was the smallest subclone to allow pBluescript to replicate in UIA5, suggesting that this region encodes functions essential and necessary for the replication of pAtC58.

Analysis of the Nucleotide Sequence of the 4.3-kb Fragment in pBS-X2

The DNA nucleotide sequence of the 4.3-kb region, which is necessary for the replication of ColE1 derivatives in UIA5, was determined. The region contains three open reading frames (ORFs), which orient from right to left in the diagram shown in Fig. 1B. These three ORFs are closely related to the replication genes *repA*, *repB*, and *repC* from pTiC58 in the deduced amino acid sequence level (Table 2). The *rep* genes of pAtC58 are also similar to those of pTi-SAKURA [36], pTiB6S3 [37], and pRiA4b, the Ri plasmid from *A. rhizogenes* A4 [23]. Therefore, we named these three ORFs of pAtC58 as *repA*, *repB*, and *repC*, respectively. The *rep* genes of pAtC58 are also similar to those of several plasmids in *Rhizobium* spp.; pNGR234a (the Sym plasmid from *Rhizobium* strain NGR234), pRL8JI (a cryptic plasmid from *Rhizobium leguminosarum* 3841), and p42a, b, and p42d (the Sym plasmids from *Rhizobium etli* CFN42) [10, 13, 27, 38] (Table 2). In addition to *rep* genes from the family Rhizobiaceae, the three genes of pAtC58 are also related to three genes encoded by pTAV1, a large cryptic plasmid from *Paracoccus versutus* UW1 [6], which does not belong to the family Rhizobiaceae.

Characterization of Replication Capabilities of pBS-X2

The sequence alignments of *igs* (intergenic sequence) regions of linear chromosome from *tumefaciens* and nine plasmids from the family Rhizobiaceae showed that the *igs* region of pAtC58 is highly homologous to other plasmids (Fig. 2). The *igs* regions are well conserved among the *rep* family. It has been suggested that the *igs* region (also called *incα*) between *repB* and *repC* plays a role as a *cis*-acting element for the initiation of replication and also for a proper partitioning [28, 37, 38]. To assess the involvement of each of the *rep* genes and the putative *cis*-acting element of pAtC58 in replication and maintenance of a plasmid, several deletion derivatives and restriction fragments of pBS-X2 were subcloned in pBluescript SK(−) and transformed into UIA5.

The plasmid pBS-Rep, which contains three *rep* genes but lack the region downstream to *repC*, was maintained in UIA5, but less stable than pBS-X2 was, suggesting that the region downstream to *repC* somehow contributes to the the stability of the plasmid. Previous report showed that, in the symbiotic plasmid in *Rhizobium* strains, the region downstream to *repC*, called *incβ*, is necessary for replication and partitioning of the plasmid [28, 35]. Another study about the region downstream of *repC* showed that RepB

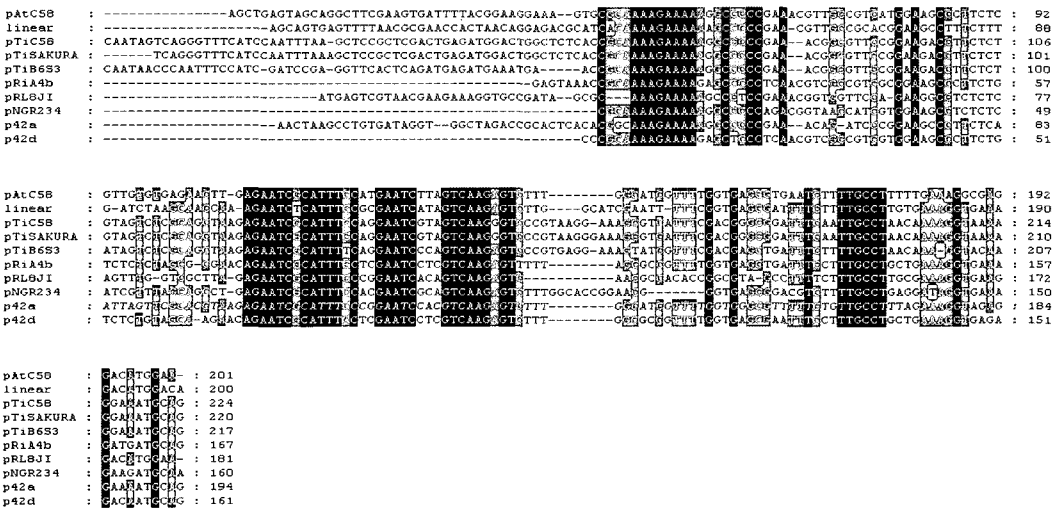

Fig. 2. Sequence alignment of *igs* from *rep* regions of nine plasmids from the family Rhizobiaceae.
These plasmids contain *repABC*-type replication systems. Highly identical sequences are shadowed.

protein binds this region and constitutes a functional system for partitioning and incompatibility of each replicon [7, 35]. It is likely that such a *cis*-acting element is not completely present in pBS-Rep. The plasmid pBS-ΔpRep could not be maintained in UIA5. This clone must lack a promoter for the *rep* operon.

Deletions in one of the *repA* and *repB* genes still conferred the carbenicillin resistance on UIA5. However, defects in the *repA* or *repB* genes affected the ability to allow a stable maintenance of the plasmid in UIA5. After 48 h of incubation without selection, only 6% and 12% of the colonies, respectively, sustained the plasmid, suggesting that these two genes are not essential for replication, but instead necessary for the stability of plasmid maintenance or for an efficient replication. In consistence with our results, previous study showed that the lack of RepA and RepB products severely decreases plasmid stability, suggesting that these genes are by themselves not essential for replication, but required for an efficient partitioning [6, 23, 28, 37]. Therefore, together with these previous reports, our result suggests that *repA* and *B* are necessary for the stable maintenance of the plasmid. Alternatively, it is also possible that a proper partitioning of the plasmid requires functions encoded by the two genes.

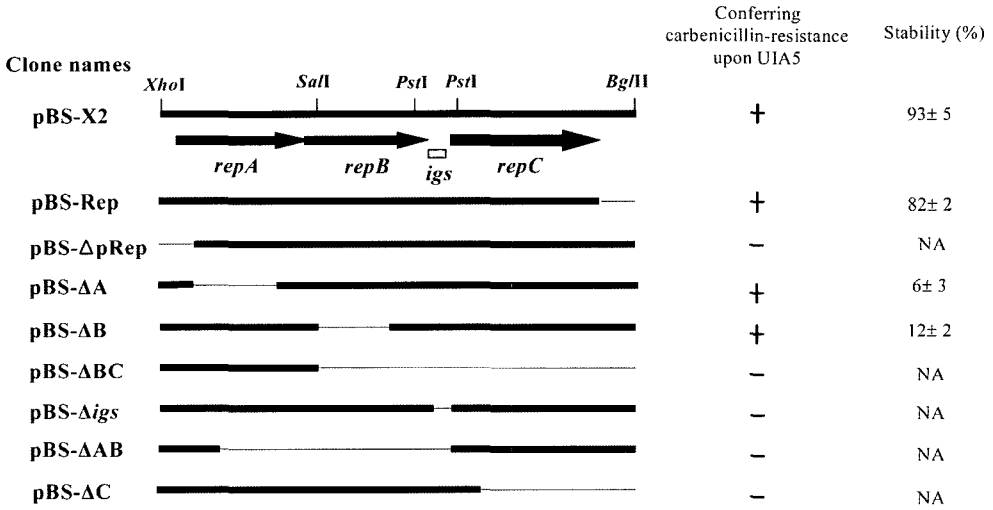

Fig. 3. Genetic map of subclones from pBS-X2, and the ability and stability of each subclone to allow pBluescript derivatives to replicate in UIA5.
Stabilities of each subclone were measured by examining the ability to confer cabenicillin resistance on UIA5 and also by plasmid preparation after 48 h of incubation without selection. Stability of maintenance in UIA5 was expressed by the percentage of cells harboring plasmids after consecutive culture of cells without antibiotic selection. NA, not applicable.

Clones with deletions in the region containing *igs* or *repC* (clones harboring pBS-ΔBC, pBS-Δigs, pBS-ΔABi, or pBS-ΔC in Fig. 3) completely lost the ability to reside in UIA5, suggesting that these regions are absolutely essential for the replication of the plasmid. This result agrees with a previous report that *repC* genes of pTiC58 and pSym plasmids are essential for replication and believed to code for replication initiation proteins [14, 28]. Furthermore, some plasmids, such as pRmeGR4a from *R. melioti* GR4, encode a RepC homolog as an initiator protein, but does not contain genes related to *repA* or *repB* [21]. This suggests that RepC is essential for replication of plasmids, and that RepA and RepB play some auxiliary roles in replication and maintenance of plasmids.

The Expression of *rep* Genes in pAtC58 is Not Induced by a Quorum-Sensing Signal

The expressions of *rep* genes in pTiC58 are induced by autoinducer signal molecules responsible for quorum sensing [20, 25]. We tested whether the expressions of *rep* genes in pAtC58 were also induced by the AAI molecule by measuring the β-galactosidase activities from *lacZ* fusions in each *rep* gene upon the induction with AAI. The result showed that the AAI molecule did not affect the expression of any of the *rep* genes in pAtC58 (Fig. 4). β-Galactosidase activities of those reporter fusions in bigger clones comprising not only the *rep* region but also regions downstream and upstream to the region were also not induced by AAI (data not shown). These indicated that, unlike pTiC58, the expression of *rep* genes in pAtC58 is not dependent on quorum sensing mediated by the AAI molecule. Although they both contain homologous *rep* genes, this suggests that the plasmids pAtC58 and pTiC58 are distinct in their regulation of the expression of the genes.

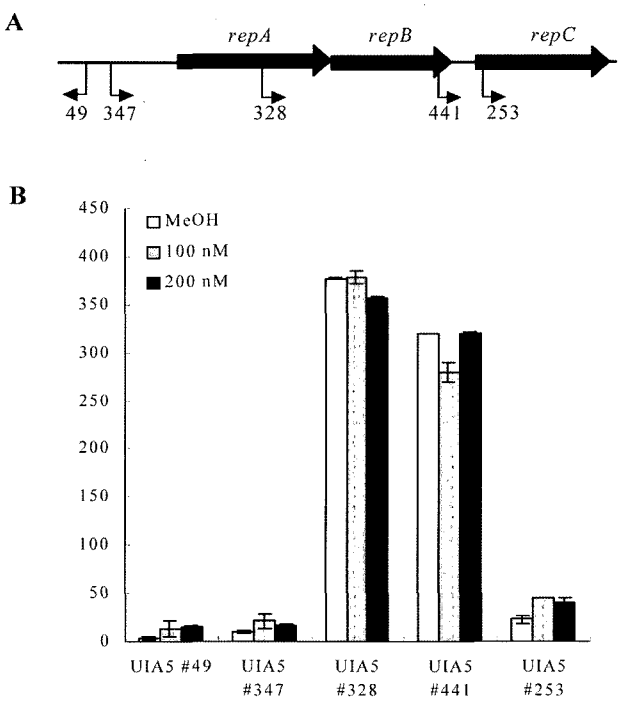

Fig. 4. Expression of *rep* genes in pAtC58 using *lacZ*-fusions on pCH-1 generated by Tn*3*HoHoI.
A. Genetic map of Tn*3*HoHoI-insertion derivatives of pCH-1 (2). Insertion #49 and #347 were mapped in the region upstream to the *rep* operon. B. β-Galactosidase activities of Tn*3*HoHoI-insertion derivatives of pCH-1 in the presence and absence of the synthetic AAI molecule at final concentrations of 100 nM (shadowed bars) and 200 nM (dark bars) dissolved in methanol.

The *rep* Region in pAtC58 is Phylogenically Closer to Those of Ri Plasmids Than to Those of Ti Plasmids

For proper maintenance as well as replication of plasmids, functional *cis*- and *trans*-acting elements are essential.

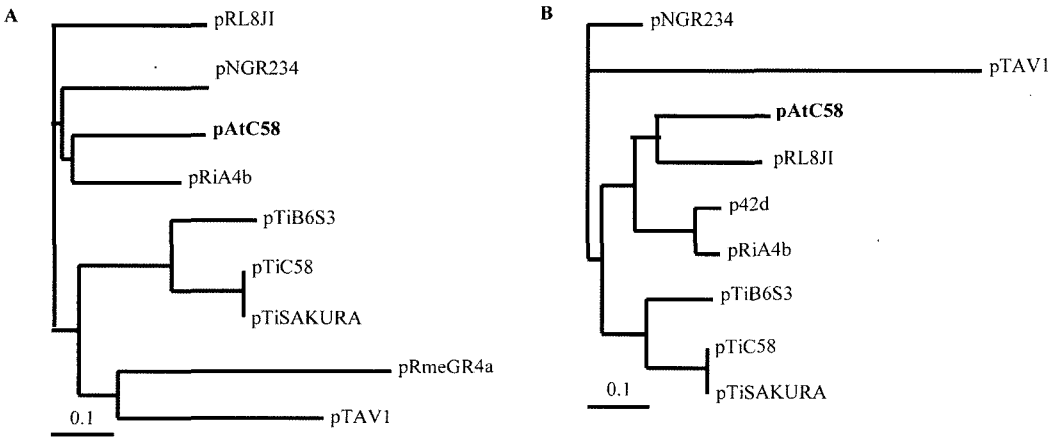

Fig. 5. Phylogenic relationships between the *cis*- and *trans*-elements of *repABC*-type replicators.
The unrooted phylogenic trees for RepC amino acid sequences (A) and the *igs* regions (B) are presented. The plasmid pRmeGR4a encodes a RepC homologue, but does not contain genes related to *repA* or *repB* [21].

Certain genetic defects in those elements result in loss of a whole set of genes encoded by the plasmids. Therefore, such elements are expected to be well conserved, compared with other less essential genetic elements in plasmids. Therefore, nucleotide sequences of those elements from various plasmids can serve as good sources to unravel the relationship among those plasmids.

Phylogenic analysis of *repC* and the *igs* regions showed that these regions from pAtC58 are closely clustered with those from pRi plasmids and from sym plasmids in *Rhizobium* sp. In contrast, pTiC58 is closely related to those of other Ti plasmids. This result suggests that pAtC58 and pTiC58 are phylogenically secluded from each other, and that it is likely that these two plasmids evolved from distinct origins. It is quite possible that *Agrobacterium* cells obtained the cryptic plasmid originated from another related bacterium, which belongs to a *Rhizobium* spp., by a horizontal transfer in the rhizosphere, as observed commonly among soil microorganisms [19].

RepC is responsible for the incompatibility of plasmids as well as for replication initiation [14]. Divergence in RepC may also be responsible for the compatibility properties marked by many members of plasmids carrying *rep* genes. However, it has also been reported that two plasmids containing *repC* genes belonging to the same group coexist in a field isolate of *R. leguminosarum* bv. viciae [30]. Therefore, in addition to RepC, some other functions may also be involved in the incompatibility properties in the *repABC*-type family plasmid. Tabata *et al.* [37] reported that a clone containing only the *igs* region exerted incompatibility against an IncRh1 Ti plasmid, suggesting that the region is essential for incompatibility. The phylogenic distance between *repC* and *igs* in pAtC58 and those in pTiC58 suggests that these two plasmids contain distinct *trans*- and *cis*-acting elements, which together contribute to the compatibility of the plasmids, and therefore, these two plasmids can coexist in the same cell.

To the best of our knowledge, this is the first study of the replication region from the cryptic plasmid of an *Agrobacterium* spp. Functions of the gene products and *cis*-acting elements in the *rep* operon of pAtC58 are now being studied. The study of the *rep* region of pAtC58 may provide clues to elucidate the evolutionary relationships among plasmids in bacteria belonging to Rhizobiaceae. Furthermore, using this replication region, the construction of a low-copy number cloning vector feasible for a genetic study of *Agrobacterium* spp. would be possible.

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References

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.
2. Baek, C.-H., S. K. Farrand, K. E. Lee, D. K. Park, J. K. Lee, and K.-S. Kim. 2003. Convergent evolution of amadori opine catabolic systems in plasmids of *Agrobacterium tumefaciens*. *J. Bacteriol.* **185:** 513–524.
3. Baek, C.-H., S. K. Farrand, D. K. Park, K. E. Lee, W. Hwang, and K.-S. Kim. 2005. Genes for utilization of deoxyfructosyl glutamine (DFG), an amadori compound, are widely dispersed in the family Rhizobiaceae. *FEMS Microbiol. Ecol.* **53:** 221–233.
4. Baek, C.-H. and K.-S. Kim. 2003. *lacZ*- and *aph*-based reporter vectors for *in vivo* expression technology. *J. Microbiol. Biotechnol.* **13:** 872–880.
5. Baek, C.-H., D. K. Park, K. E. Lee, W. Hwang, I. H. Kim, J. Maeng, and K.-S. Kim. 2004. Genes for the catabolism of deoxyfructosyl glutamine in pAtC58 are attributed to utilization of octopine in *Agrobacterium tumefaciens* strain NT1. *J. Microbiol. Biotechnol.* **14:** 822–828.
6. Bartosik, D., J. Baj, and M. Wlodarczyk. 1998. Molecular and functional analysis of pTAV320, a *repABC*-type replicon of the *Paracoccus versutus* composite plasmid pTAV1. *Microbiology* **144:** 3149–3157.
7. Bartosik, D., M. Szymanik, and M. Wlodarczyk. 2001. Identification of the partitioning site within the *repABC*-type replicon of the composite *Paracoccus versutus* plasmid pTAV1. *J. Biotechnol.* **183:** 6234–6243.
8. Bignell, C. and C. M. Thomas. 2001. The bacterial ParA-ParB partitioning proteins. *J. Biotechnol.* **91:** 1–34.
9. Cangelosi, G. A., E. A. Best, G. Martinetti, and E. W. Nester. 1991. Genetic analysis of *Agrobacterium*. *Methods Enzymol.* **204:** 384–397.
10. Cevallos, M., H. Porta, J. Izquierdo, C. Tun-Garrido, A. García-de-los-Santos, G. Dávila, and S. Brom. 2002. *Rhizobium etli* CFN42 contains at least three plasmids of the *repABC* family: A structural and evolutionary analysis. *Plasmid* **48:** 104–116.
11. del Solar. G., R. Giraldo, M. J. Ruiz-Echevarría, M. Espinosa, and R. Díaz-Orejas. 1998. Replication and control of circular bacterial plasmids. *Microbiol. Mol. Biol. Rev.* **June:** 434–464.
12. Dessaux, Y., J. Tempé, and S. K. Farrand. 1987. Genetic analysis of mannityl opine catabolism in octopine-type *Agrobaterium tumefaciens* strain 15955. *Mol. Gen. Genet.* **208:** 301–308.
13. Freiberg, C., R. Fellay, A. Bairoch, W. J. Broughton, A. Rosenthal, and X. Perret. 1997. Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature (London)* **387:** 394–401.

14. Gallie, D. R., M. Hagiya, and C. I. Kado. 1985. Analysis of *Agrobacterium tumefaciens* plasmid pTiC58 replication region with a novel high-copy-number derivative. *J. Bacteriol.* **161:** 1034–1041.
15. Jouanin, L. 1984. Restriction map of an agropine-type Ri plasmid and its homologies with Ti plasmids. *Plasmid* **12:** 91–102.
16. Keen, N. T., S. Tamaki, D. Kobayashi, and D. Trollinger. 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. *Gene* **70:** 191–197.
17. Kho, D.-H., J.-H. Jang, H.-S. Kim, K.-S. Kim, and J. K. Lee. 2003. Quorum sensing of *Rhodobacter sphaeroides* negatively regulates cellular poly-β-hydroxybutyrate content under aerobic growth conditions. *J. Microbiol. Biotechnol.* **13:** 477–481.
18. Kim, K.-S. and S. K. Farrand. 1996. Ti plasmid-encoded genes responsible for catabolism of the crown gall opine mannopine by *Agrobacterium tumefaciens* are homologs of the T-region genes responsible for synthesis of this opine by the plant tumor. *J. Bacteriol.* **178:** 3275–3284.
19. Kim, T., M.-S. Kim, M.-K. Jung, M.-J. Joe, J.-H. Ahn, K.-H. Oh, M. Lee, M. Kim, and J.-O. Ka. 2005. Analysis of plasmid pJP4 horizontal transfer and its impact on bacterial community structure in natural soil. *J. Microbiol. Biotechnol.* **15:** 376–383.
20. Li, P.-L. and S. K. Farrand. 2000. The replicator of the nopaline-type Ti plasmid pTiC58 is a member of the *repABC* family and is influenced by the TraR-dependent quorum-sensing system. *J. Bacteriol.* **182:** 179–188.
21. Mercado-Blanco, J. and J. Olivares. 1994. The large nonsymbiotic plasmid pRmeGR4a of *Rhizobium melioti* GR4 encodes a protein involved in replication that has homology with the RepC protein of *Agrobacterium* plasmids. *Plasmid* **32:** 75–79.
22. Nair, G. R., Z. Liu, and A. N. Binns. 2003. Reexamining the role of the accessory plasmid pAtC58 in the virulence of *Agrobacterium tumefaciens* strain C58. *Plant Physiol.* **133:** 989–999.
23. Nishiguchi, R., M. Takanami, and A. Oka. 1987. Characterization and sequence determination of the replicator region in the hairy-root-inducing plasmid pRiA4b. *Mol. Gen. Genet.* **206:** 1–8.
24. Palmer, K. M., S. L. Turner, and J. P. W. Young. 2000. Sequence diversity of the plasmid replication gene *repC* in the *Rhizobiaceae*. *Plasmid* **44:** 209–219.
25. Papas, K. M. and S. C. Winans. 2003. A *luxR*-type regulator from *Agrobacterium tumefaciens* elevates Ti plasmid copy number by activating transcription of plasmid replication genes. *Mol. Microbiol.* **48:** 1059–1073.
26. Papas, K. M. and S. C. Winans. 2003. The RepA and RepB autorepressors and TraR play opposing roles in the regulation of a Ti plasmid *repABC* operon. *Mol. Microbiol.* **49:** 441–455.
27. Ramírez-Romero, M. A., P. Bustos, L. Girard, O. Rodríguez, M. A. Cevallos, and G. Dávilla. 1997. Sequence, localization and characteristics of the replicator region of the symbiotic plasmid of *Rhizobium etli*. *Microbiology* **143:** 2825–2831.
28. Ramírez-Romero, M. A., N. E. Soberón, A. Perez-Oseguera, J. M. Téllez-Sosa, and M. A. Cevallos. 2000. Structural elements required for replication and incompatibility of the *Rhizobium etli* symbiotic plasmid. *J. Bacteriol.* **182:** 3117–3124.
29. Ramírez-Romero, M. A., J. Téllez-Sosa, H. Barrios, A. Perez-Oseguera, V. Rosas, and M. A. Cevallos. 2001. RepA negatively autoregulates the transcription of the *repABC* operon of the *Rhizobium etli* symbiotic plasmid basic replicon. *Mol. Microbiol.* **42:** 195–204.
30. Rigottier-Gois, L., S. L. Turner, J. P. W. Young, and N. Amarger. 1998. Distribution of *repC* plasmid-replication sequences among plasmids and isolates of *Rhizobium leguminosarum* bv. *viciae* from field populations. *Microbiology* **144:** 771–780.
31. Rosenberg, C. and C. Huguet. 1984. The pAtC58 plasmid of *Agrobacterium tumefaciens* is not essential for tumor induction. *Mol. Gen. Genet.* **196:** 533–536.
32. Sambrook, J. and D. W. Russell. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., U.S.A.
33. Sciaky, D., A. L. Montoya, and M. D. Chilton. 1978. Fingerprints of *Agrobacterium* Ti plasmids. *Plasmid* **1:** 238–253.
34. Simon, R., U. Priefer, and A. Pühler. 1983. A broad host range mobilization for *in vivo* genetic engineering: Transposon mutagenesis in gram-negative bacteria. *Biotechnology* **NOV:** 784–791.
35. Soberón, N., T. Venkova-Canova, M. A. Ramírez-Romero, J. Téllez-Sosa, and M. A. Cevallos. 2004. Incompatibility and the partitioning site of the *repABC* basic replicon of the symbiotic plasmid from *Rhizobium etli*. *Plasmid* **51:** 203–216.
36. Suzuki, K., N. Ohta, Y. Hattori, M. Uraji, A. Kato, and K. Yoshida. 1998. Novel structural difference between nopaline- and octopine-type *trbJ* genes: Construction of genetic and physical map and sequencing of *trb/traI* and *rep* gene clusters of a new Ti plasmid pTi-SAKURA. *Biochem. Biophys. Acta* **1396:** 1–7.
37. Tabata, S., P. J. J. Hooykaas, and A. Oka. 1989. Sequence determination and characterization of the replicator region in the tumor-inducing plasmid pTiB6S3. *J. Bacteriol.* **171:** 1665–1672.
38. Turner, S. L. and J. P. W. Young. 1995. The replicator region of the *Rhizobium leguminosarum* cryptic plasmid pRL8JI. *FEMS Microbiol. Lett.* **133:** 53–58.
39. Van Larebeke, N., G. Engeler, M. Holsters, S. Van den Elsacker, I. Zaenen, R. A. Shilperoort, and J. Shell. 1974. Large plasmid in *Agrobacterium tumefaciens* essential for crown gall-inducing ability. *Nature* **252**: 169–170.
40. Van Montagu, M. and J. Shell. 1979. *The Plasmid of Agrobacterium tumefaciens*. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.
41. Zhang L., P. J. Murphy, A. Kerr, and M. E. Tate. 1993. *Agrobacterium* conjugation and gene regulation by *N*-acyl-L-homoserine lactones. *Nature* **362:** 446–448.