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The RepFIIA replicon of the natural *Escherichia coli* plasmid pRK100

Replikon RepFIIA naravnega plazmida pRK100 bakterije *Escherichia coli*

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Abstract. The aim of the presented study was to identify the similarity of the plasmid pRK100 RepFIIA replicon (replication region) with similar replicons of other known plasmids of *Enterobacteriaceae*. For this purpose, within the determined nucleotide sequence of pRK100, the RepFIIA replicon *rep* genes/regions were identified. The nucleotide sequences of the pRK100 determined *rep* genes/regions were subsequently compared with the nucleotide sequences of other RepFIIA replicon *rep* genes/regions deposited in GenBank. Further, the nucleotide divergence between them was calculated. The obtained results clearly demonstrated, that the individual pRK100 *rep* regions are the same/most similar to *rep* regions from different plasmids. *RepA2* of pRK100 is most similar to *repA2* of pCP301, pINV_F6_M1382, pWR501 and R1, *copA* is the same as *copA* of plasmids pC15-1a and R100, *repA6* of pRK100 is the same as *repA6* in plasmids pC15-1a, pCP301, pINV_F6_M1382, pWR501, R1 and R100, *repA1* is most similar to *repA1* of the plasmid p1658/79, and *repA4* of pRK100 is most similar to *repA4* of pC15-1a. Hence, the composition of the pRK100 RepFIIA replicon is mosaic and unique among the plasmids.

Key words: plasmid, RepFIIA replicon, *Enterobacteriaceae*, nucleotide divergence

Izvleček. Cilj raziskave je bil označiti podobnost replikona (replikacijske regije) RepFIIA z drugimi podobnimi replikoni znanih plazmidov enterobakterij. V ta namen smo v nukleotidnem zaporedju replikona RepFIIA plazmida pRK100 poiskali posamezne gene/regije *rep* in njihovo nukleotidno zaporedje primerjali z drugimi, deponiranimi, nukleotidnimi zaporedji RepFIIA ter izračunali nukleotidno divergenco. Dobljeni rezultati so jasno pokazali, da so različni geni/regije *rep* v replikonu RepFIIA plazmida pRK100 enaki/zelo podobni *rep* različnih plazmidov. *RepA2* od pRK100 je najbolj podoben genu *repA2* plazmidov pCP301, pINV_F6_M1382, pWR501 in R1, *copA* je enak genu *copA* na plazmidih pC15-1a in R100, regija *repA6* plazmida pRK100 je enaka regiji *repA6* plazmidov pC15-1a, pCP301, pINV_F6_M1382, pWR501, R1 in R100, gen *repA1* je najbolj podoben genu *repA1* plazmida p1658/79, in regija *repA4* plazmida pRK100 je najbolj podobna regiji *repA4* plazmida pC15-1a. Povzamemo lahko, da je replikon RepFIIA plazmida pRK100 sestavljen kot mozaik in da ga v takšni sestavi do sedaj še niso našli na nobenem drugem plazmidu.

Ključne besede: plazmid, replikon RepFIIA, *Enterobacteriaceae*, nukleotidna divergenca

Introduction

Plasmids, extrachromosomal DNA elements, can be found in all three domains of the living world, in *Archaea*, *Bacteria* and *Eukarya* (Holmes & al. 1995, Solar & al. 1998, Zillig & al. 1998). These elements encode a remarkable array of phenotypic traits of medical, agricultural, environmental and commercial importance (Helinski & al. 1996). Encoded traits include resistances to heavy metals, supplementary metabolic pathways and pathways for degradation of xenobiotics, as well as virulence factors and resistances to antibiotics (Kado 1998). Further, plasmids can have the machinery to transfer themselves and other parts of the genome into different species, genera, or sometimes even families (Firth & al. 1996). Plasmids can also incorporate and deliver genes by recombination or transposition and by this means increase the genetic exchange in- and between bacterial populations (Solar & al. 1998).

All plasmids harbour a replicon (replication region), which is needed for the stable propagation and maintenance in the host cell. Regardless of plasmid size, the replicon of a plasmid generally consists of a contiguous set of information that includes a definable origin, where DNA replication initiates (*ori*), a structural gene encoding the plasmid-specific protein required for the initiation of replication, and one or more adjoining controlling elements. All this information is often contained within a segment that is 3 kb or less in size (Helinski & al. 1996). The replicons are designated and grouped into families (Couturier & al. 1988).

Replicons belonging to the RepFIIA family typically consist (Fig. 1) of *repA2* encoding a repressor, the *copA* gene that encodes an antisense RNA molecule, a *repA1* gene whose protein initiates plasmid replication by binding to the downstream *ori*, the *repA6* region encoding a short leader peptide, and a *repA4* region. The RepA2 repressor is assumed to regulate transcription of *repA1* mRNA, while the antisense RNA CopA which is complementary to the leader region of *repA1* mRNA (CopT), regulates translation. When CopA binds to CopT, *repA6,* which is necessary for RepA1 synthesis, is not expressed (Blomberg & al. 1992). The *repA4* appears to be important for the stability of plasmid maintenance (Jiang & al. 1993). Further it is known, that the replicons of this family are mosaic (Osborn & al. 2000), i.e. individual genes encoded in this replicon originate from different sources.

pRK100 is an ~145-kb plasmid isolated from a uropathogenic *Escherichia coli* strain and it has been to a large extent characterised. It belongs to the IncF incompatibility group and encodes two antibiotic resistances, ampicillin and tetracycline, two colicins, ColV and ColIa, and the aerobactin (*iuc*) and enterochelin (*iro*) iron uptake system. Further it was demonstrated that pRK100 harbours two different replicons, a RepFIB and RepFIIA replicon (Žgur-Bertok & al. 1990, Ambrožič & al. 1998, Starčič Erjavec, 2003). In the presented study the genes/regions of the pRK100 RepFIIA rep-

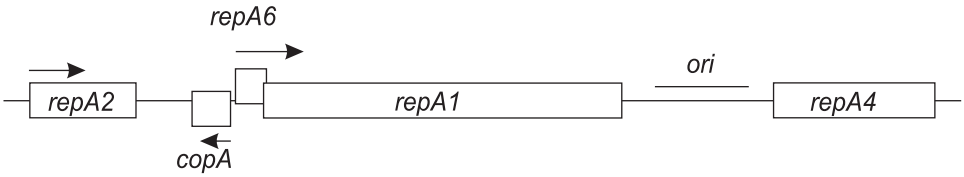

Fig. 1: Map of the RepFIIA replicon.
Genes/regions of a typical RepFIIA replicon are depicted. To clearly show the individual studied sequences, some boxes representing genes/regions are offline. The direction of mRNA transcription is also marked. *ori* is the origin of replication, where the RepA1 protein binds and starts the replication.

Slika 1: Mapa replikona RepFIIA.
Označeni so geni/regije tipičnega replikona RepFIIA. Zaradi razvidnosti lege preučevanih zaporedij, so nekateri okvirčki, ki prikazujejo gene/regije, premaknjeni. Označena je tudi smer prepisa mRNA iz posameznega gena. *ori* je regija, kjer se veže replikatorski protein RepA1 in prične s podvajanjem plazmida.

licon were analysed for their similarity with the RepFIIA replicon's genes/regions of related plasmids p1658/79, pB171, pC15-1a, pCP301, pINV_F6_M1382, pO157, pTUC100, pWR501, R1 and R100. The nucleotide divergence between pRK100 and the other plasmids was determined. The results of our study show that the RepFIIA replicon of pRK100 is mosaic and unique in its composition.

Method

Sequence analysis for open reading frames (ORF)

The determined RepFIIA replicon nucleotide sequence, 2159 bp in length, (GenBank accession number AY234375) was analyzed for open reading frames with the help of the program "ORF Finder" available on the web site http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi.

Sequence analysis for DNA similarity

The program Nucleotide-nucleotide BLAST (Altschul & al. 1997) available on the web site http://www.ncbi.nlm.nih.gov/BLAST/ was used to search for nucleotide sequences similar to the pRK100 RepFIIA nucleotide sequence. The DNA sequences of *rep* genes/regions were compiled and analysed using the CLUSTAL W (Thompson & al. 1994) program for sequence alignment. The program DNADIST in the PHYLIP package (Felsenstein 1993, Felsenstein 1989) was used for calculating distance matrixes.

Results

Genes/regions of the RepFIIA nucleotide sequence of pRK100

The pRK100 RepFIIA replicon has been deposited in GenBank under the Accession Number AY234377. In this deposited nucleotide sequence, the RepFIIA replicon is harboured from Nt 1401 to Nt 3559. To identify the *rep* genes/regions in the deposited nucleotide sequence of the pRK100 RepFIIA replicon, the internet program "ORF Finder" was used. To further define the *rep* genes/regions, the pRK100 RepFIIA sequence was compared to other known *rep* genes/regions of similar replicons. The RepFIIA replicon was found to harbour *repA2*, *copA*, *repA6*, *repA1*, and *repA4* sequences (Tab. 1).

Table 1: Predicted genes/regions in the pRK100 RepFIIA replicon (AY234377).
Tabela 1: Predvideni geni/regije replikona RepFIIA (AY234377) plazmida pRK100.

Assumed gene/region	Frame	From (bp)	To (bp)	Length
repA2	+3	1401	1661	261
copA	+1 C	1874	1782	93
repA6	+2	1886	1960	75
repA1	+3	1953	2810	858
repA4	+2	3173	3556	384

A pRK100 RepFIIA-like replicon can be found on many other plasmids

With the goal to find plasmids with similar replicons, which could be compared with the pRK100 RepFIIA replicon, a BLAST search with the nucleotide sequence of pRK100's RepFIIA was performed. The search revealed that many other plasmids carry similar replicons (Tab. 2). The most similar RepFIIA replicon was harboured by the *Escherichia coli* plasmid p1658/9. Most of the plasmids with similar replicon sequences were harboured on plasmids hosted by *Escherichia coli* or

Shigella flexneri however, some plasmids with less similar sequences were harboured also by other enteric bacterial species, as *Klebsiella pneumoniae*, *Shigella sonnei* and *Salmonella* Typhimurium. All plasmids exhibiting similarity with the pRK100 RepFIIA replicon belong to the broad RepFIIA family of replicons, those with higher similarity are members of the same Inc group – the IncFII, and plasmids with lower similarity belong to other Inc groups (IncFIA, IncFIC, IncFIV,..)

Table 2: BLAST hits for the nucleotide sequence of the pRK100 RepFIIA replicon with a score higher than 200.
Tabela 2: Zadetki z BLAST z rezultatom več kot 200 bitov za replikon RepFIIA plazmida pRK100.

GenBank Acc. Number	**Plasmid**	**Host**	**BLAST score (bits)**
AF550679	p1658/9	*Escherichia coli*	3733
V00351	R1(RSC13)	*Escherichia coli*	3527
AF177050	pWR100	*Shigella flexneri*	3213
AL391753	pWR100	*Shigella flexneri*	3166
AF348706	pWR501	*Shigella flexneri*	3166
AF386526	pCP301	*Shigella flexneri* 2a strain 301	3152
AY458016	pC15-1a	*Escherichia coli*	3019
AP000342	R100	*Shigella flexneri* 2b strain 222, *Escherichia coli*	2948
AY206448	pINV_F6_M1382	*Shigella flexneri*	2843
AB011549	pO157	*Escherichia coli*	2773
AB024946	pB171	*Escherichia coli*	2759
AY091607	pTUC100	*Escherichia coli*	2746
M26937	pSU316	*Escherichia coli*	2627
V00318	R6-5	*Escherichia coli*	926
M13472	ColV2-K94	*Escherichia coli*	924
X55895	pSU212	*Escherichia coli*	759
M93064	pEI545	*Klebsiella pneumoniae*	702
AP001918	F	*Escherichia coli*	420
M16167	P307	*Escherichia coli*	414
M28098	pSU221	*Escherichia coli*	394
M27528	R124	*Escherichia coli*	339
AP005147	R64	*Salmonella typhimurium*	311
AB021078	ColIb-P9	*Shigella sonnei* (*E. coli*)	303
K02675	pCG86	*Escherichia coli*	274
AP002527	R721	*Escherichia coli*	230

The pRK100 RepFIIA replicon is mosaic and unique in its composition

In order to identify the similarity of the pRK100 RepFIIA *rep* genes/regions with other known *rep* genes/regions the computer programs CLUSTAL W and DNADIST were used. With these programs nucleotide sequences of *rep* genes/regions of pRK100 with *rep* sequences of other similar RepFIIA replicons, which were found with BLAST search and gave a BLAST score of more then 1000 bits, were compiled. For the compilation only those BLAST hits that had a complete RepFIIA replicon nucleotide sequence deposited were used, so that entire RepFIIA replicon could be compared. For the comparison of RepFIIA genes/regions harboured by different plasmids the original genes/regions, if denoted by the submitters of the sequence, were used, otherwise the genes/regions in the deposited sequence were searched for using BLAST (Tab. 3). All together the nucleotide sequences of RepFIIA replicons of 10 plasmids (p1658/79, pB171, pC15-1a, pCP301, pINV_F6_M1382, pO157, pTUC100, pWR501, R1 and R100) were compared with the nucleotide sequence of pRK100 RepFIIA replicon.

The first region analysed in the RepFIIA replicon is the *repA2* gene, encoding a repressor protein, which by binding to the promoter represses the synthesis of *repA1* mRNA (Vanooteghem & Cornelis 1990). The *repA2* gene is in plasmids pRK100, p1658/79, pCP301, pINV_F6_M1382, pWR501, and R1 261 bp long, in plasmids R100, pO157, pC15-1a and pTUC100 it is 255 bp long and in the plasmid

Table 3: Plasmids and their *rep* sequences used in CLUSTAL W and DNADIST computer programs.
The studied sequences are denoted either according to the data in GenBank or marked as BLAST to show that the sequence was searched for by BLAST. For each studied sequence the position on the deposited sequence and the length are given.

Tabela 3: Plazmidi in njihova zaporedja *rep*, ki smo jih uporabili v računalniških programih CLUSTAL W in DNADIST.
Preučevana zaporedja so označena tako kot so podana v podatkovni bazi GenBank oziroma označena z BLAST, v primeru če so bila poiskana z BLAST. Za vsako preučevano zaporedje je navedena njegova pozicija na deponiranem zaporedju in njegova dolžina.

	repA2	***length (bp)***	***copA***	***length (bp)***	***repA6***	***length (bp)***	***repA1***	***length (bp)***	***repA4***	***length (bp)***
p1658/79	BLAST: 37469–37729	261	BLAST: *c* 37850–37943	94	repA6: 37955–38029	75	repA1: 38011–38879	869	repA4: 39243–39449	207
pB171	copB: *c* 39420–39668	249	BLAST: *c* 39185–39278	94	BLAST: 39099– 39173	75	repA1: *c* 38249–39106	858	BLAST: *c* 37500–37887	388
pC15–1a	repA2: 88558–88812	255	BLAST: *c*88946–89038	93	repA6: 89050–89124	75	repA1: 89117–89974	858	repA4: 90337–90723	387
pCP301	repB: 208897–209157	261	BLAST: *c* 209278–209369	92	tapA: 209381–209455	75	repA: 209436–210305	870	BLAST: 210668–211049	383
pINV_F6–_M1382	repB: 831–1091	261	BLAST: *c* 1212–1303	92	tap: 1315–1389	75	repA: 1382–2239	858	BLAST: 2602–2988	387
pO157	repA2: 71999–72253	255	BLAST: *c* 72387–72477	91	BLAST: 72489–72563	75	repA1: 72556–73416	858	BLAST: 73775–74158	384
pTUC100	repA2: *c*5976–6230	255	repA3: 5744–5872	129	repA6: *c* 5665–5739	75	repA1: *c* 4815–5672	858	repA4: *c* 4066–4452	387
pWR501	repA2: 208603–208863	261	S0294 (inc–RNA): *c* 208980–209069	90	repA6: 209087–209161	75	repA1: 209142–210011	870	BLAST: 210374–210756	382
R1	repA2: 442–702	261	BLAST: *c* 823–915	93	BLAST: 927–1001	75	repA1: 994–1851	858	repA4: 2214–2600	387
R100	repA2: 88253–88507	255	inc: *c* 88641–88733	93	repA6: 88745–88819	75	repA1: 88812–89669	858	repA4: 90032–90418	387

pB171 it is only 249 bp long. The sequence analysis showed, that pRK100 *repA2* is most similar to *repA2* of pCP301, pINV_F6_M1382, pWR501 and R1, since the phylogenetic distance between them is the smallest, namely 0,0038 (Fig. 2A).

The second region analysed in the RepFIIA replicon is the *copA* gene, encoding the antisense RNA regulating the translation of *repA1* mRNA (Vanooteghem & Cornelis 1990). The *copA* gene is in most of the compared plasmids approximately 90 bp long. The only exception is the *copA* gene of the plasmid pTUC100, which is 129 bp long. The sequence analysis showed, that there is no nucleotide divergence between pRK100 *copA* and the copA genes of R100 and pC15-1a (Fig. 2B), and hence the pRK100 *repA* gene is identical to the appropriate genes in R100 and pC15-1a.

The third region analysed in the RepFIIA replicon is the *repA6*. *repA6* encodes a short leader peptide, whose expression is inhibited by CopA binding, preventing translation of RepA and consequently preventing plasmid replication. (Bloomberg & al. 1992). The *repA6* is only 75 nucleotides long and it has the same size in all compared plasmids. The compared nucleotide sequences of pRK100 *repA6* was completely identical (no nucleotide divergence) to *repA6* of plasmids pC15-1a, pCP301, pINV_F6_M1382, pWR501, R1 and R100 (Fig. 2C).

The fourth region analysed in the RepFIIA replicon is *repA1* gene, encoding the RepA protein needed for the plasmid's replication (Helinski & al. 1996). The *repA1* genes of plasmids pB171, pC15-1a, pINV_F6_M1382, pO157, pTUC100, R1 and R100 are 858 bp long, the *repA1* of p1658/79 is 869 bp long, and the *repA1* genes of plasmids pCP301 and pWR501 are 870 bp long. The phylogenetic distance between the pRK100 *repA1* nucleotide sequence and the *repA1* of plasmid p1658/79 is the smallest, only 0,0154 (Fig. 2D), hence the pRK100 *repA1* is most similar to *repA1* of plasmid p1658/79.

The fifth and the last region analysed in the RepFIIA replicon, is *repA4*. Though it encodes no product, it is important for the stability of plasmid maintenance (Jiang & al. 1993). The p1658/79 *repA4* is 207 bp long, while the *repA4* sequences of other compared plasmids are around 385 bp long. The phylogenetic distance between the pRK100 *repA4* nucleotide sequence and *repA4* of plasmid pCP15-1a is the smallest, 0,0520 (Fig. 2E), therefore the *repA4* nucleotide sequence of pRK100 is most similar to *repA4* of plasmid pCP15-1a.

Since the individual pRK100 *rep* genes/regions are similar to *rep* genes/regions of different plasmids, it can be concluded, that the pRK100 RepFIIA replicon is mosaic in structure and unique among the RepFIIA replicons.

Discussion

Plasmid replicons are essential for plasmid maintenance in the host cell. The replicons can differ with regard to their replication control mechanisms, origin of replication sequences and replication proteins. Plasmids with very similar origin sequences and replication control mechanisms are assigned into families. The pRK100 replicon described in this article belongs to the RepFIIA family of replicons.

In order to characterise the RepFIIA replicon of pRK100 its nucleotide sequence was first searched for genes/regions. Each RepFIIA replicon consists of five genes/regions, *repA2* gene, *copA* gene, *repA6* region, *repA1* gene and *repA4* region. With the help of the computer programs "ORF Finder" and BLAST the five genes/regions in the pRK100 RepFIIA replicon were determined, however the genes are only putative and more experimental work is needed to confirm the predicted gene lengths and their functions.

To elucidate the similarity of the pRK100 RepFIIA replicon with other known RepFIIA replicons, a BLAST search on the complete pRK100 RepFIIA nucleotide sequence was performed. More then 170 BLAST hits were found, most of them belonging to replicon sequences of plasmids from *Enterobacteriaceae.* This is not surprising as the broad RepFIIA replicon family is known to be highly prevalent in enteric bacteria (Osborn & al. 2000).

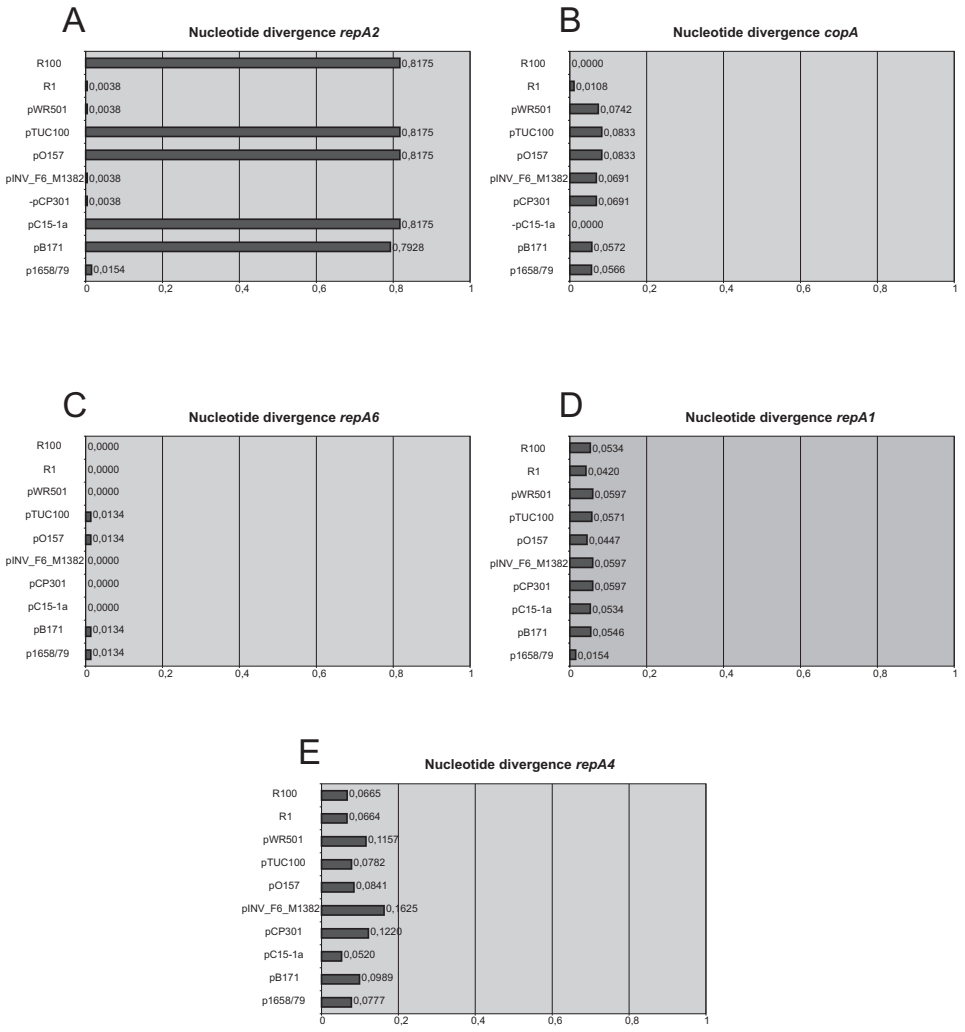

Fig. 2: Nucleotide divergence of pRK100 RepFIIA *rep* gene/region sequences and related sequences.
Nucleotide divergence, as the measurement of the phylogenetic nucleotide distance, was calculated according to the Kimura-2 parameter. Nucleotide divergences between pRK100 sequences and sequences of other related plasmids are plotted: Panel A – nucleotide divergence of *repA2*, panel B – nucleotide divergence of *copA*, panel C – nucleotide divergence of *repA6*, panel D – nucleotide divergence of *repA1*, and panel E – nucleotide divergence of *repA4.*

Slika 2: Nukleotidna divergenca dobljenih zaporedij genov/regij *rep* replikona RepFIIA plazmida pRK100 in sorodnih zaporedij.
Nukleotidna divergenca, kot mera za filogenetsko razdaljo, je bila izračunana po parametru Kimura-2. Nukleotidne divergence med zaporedji plazmida pRK100 in ostalimi sorodnimi zaporedji so prikazana: graf A – nukleotidna divergenca *repA2*, graf B – nukleotidna divergenca *copA*, graf C – nukleotidna divergenca *repA6*, graf D – nukleotidna divergenca *repA1* in graf E – nukleotidna divergenca *repA4.*

The most relevant BLAST hits (BLAST score more then 1000 bits) were used for a detailed similarity analysis. The chosen RepFIIA replicons to be compared with the pRK100 replicon were harboured by plasmids p1658/79, pB171, pC15-1a, pCP301, pINV_F6_M1382, pO157, pTUC100, pWR501, R1 and R100. Since it is known, that the RepFIIA replication family is mosaic in its composition (Osborn & al. 2000), each *rep* region was analysed separately. Also from our analysis the mosaic structure of the pRK100 RepFIIA replicon is evident, since *RepA2* of pRK100 is most similar to *repA2* of pCP301, pINV_F6_M1382, pWR501 and R1, *copA* is the same as *copA* of plasmids pC15-1a and R100, the *repA6* of pRK100 is the same as *repA6* in plasmids pC15-1a, pCP301, pINV_F6_M1382, pWR501, R1 and R100, *repA1* is the most similar to *repA1* of plasmid p1658/79, and *repA4* of pRK100 is the most similar to *repA4* of pC15-1a. Further, it can also be concluded, that the composition of the pRK100 RepFIIA replicon is unique.

Even though all nucleotide sequences of genes/regions incorporated into this study, belong to the same replicon, the RepFIIA, the nucleotide divergence between different genes/regions is not the same, thus the *repA6* region is very conserved, while other genes, as *copA* and *repA1*, are less conserved. A higher level of nucleotide divergence was observed in the *repA4* sequence. This fact is not surprising, since this sequence has no product and hence no product-connected selection can influence the evolution of this sequence. However, the greatest differences in nucleotide divergence were observed in the *repA2* gene, encoding the repressor. It might be assumed that the observed differences are important for plasmid incompatibility and it would be very interesting to test the ability of the studied plasmids to propagate in the same host.

The observed mosaicism of the pRK100 RepFIIA replicon is not the only example of a mosaic sequence of pRK100. In a previous report (Starčič Erjavec & al. 2002) we also demonstrated, that the pRK100 *tra* region is mosaic. However, overall the studied *tra* region genes were most similar to the *tra* genes of plasmid F, but in the case of the pRK100 RepFIIA replicon, no overall similarity with only one plasmid could be detected. This again illustrates, that plasmid genes are mosaic and formed by multiple recombination events between diverse ancestral genes (Boyd & al. 1996).

Conclusions

To summarise and conclude:

1. the pRK100 RepFIIA replicon harbours 5 genes/regions *repA2*, *copA*, *repA6*, *repA1* and *repA4;*
2. the individual *rep* genes/regions of the pRK100 RepFIIA replicon exhibit different nucleotide divergence when compared with different related plasmids;
3. the pRK100 RepFIIA replicon is mosaic and unique;
4. many other plasmids of *Enterobacteriaceae* carry replicons similar to pRK100 RepFIIA replicon.

Povzetek

V predstavljeni raziskavi smo na nivoju nukleotidnih zaporedij preučevali podobnost replikona RepFIIA plazmida pRK100 z replikoni RepFIIA ostalih sorodnih plazmidov. S pomočjo računalniških programov "ORF Finder" in BLAST smo na zaporedju replikona RepFIIA plazmida pRK100 poiskali replikacijske gene/zaporedja *repA2*, *copA*, *repA6*, *repA1* in *repA4.* Z računalniškima programoma CLUSTAL W in PHYLIP smo predvidene replikacijske gene/zaporedja primerjali z replikacijskimi geni/zaporedji drugih sorodnih plazmidov (p1658/79, pB171, pC15-1a, pCP301, pINV_F6_M1382, pO157, pTUC100, pWR501, R1 in R100), ki so deponirani v GenBank in smo jih poiskali z računalniškim programom BLAST. Na podlagi dobljenih nukleotidnih divergenc je razvidno, da je gen

RepA2 od pRK100 najbolj podoben genu *repA2* plazmidov pCP301, pINV_F6_M1382, pWR501 in R1, *copA* je enak genu *copA* na plazmidih pC15-1a in R100, regija *repA6* plazmida pRK100 je enaka regiji *repA6* plazmidov pC15-1a, pCP301, pINV_F6_M1382, pWR501, R1 in R100, gen *repA1* je najbolj podoben genu *repA1* plazmida p1658/79, in regija *repA4* plazmida pRK100 je najbolj podobna regiji *repA4* plazmida pC15-1a. Če povzamemo vse rezultate, lahko zaključimo, da je replikon RepFIIA plazmida pRK100 sestavljen kot mozaik in da ga v takšni sestavi do sedaj še niso našli na nobenem drugem plazmidu.

Literature

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