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SERE, a widely dispersed bacterial repetitive DNA element

G. RAJASHEKARA, T. KOEUTH*, S. NEVILE, A. BACK, K. V. NAGARAJA, J. R. LUPSKI* and V. KAPUR

Department of Veterinary Pathobiology, University of Minnesota, St Paul, MN 55108 and *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA

The presence of a *Salmonella* serotype Enteritidis repeat element (SERE) located within the upstream regulatory region of the *sefABCD* operon encoding fimbrial proteins is reported. DNA dot-blot hybridisation analyses and computerised searches of genetic databases indicate that SERE is well conserved and widely distributed throughout the bacterial and archaeal kingdoms. A SERE-based polymerase chain reaction (SERE-PCR) assay was developed to fingerprint 54 isolates of Enteritidis representing nine distinct phage types and 54 isolates of other *Salmonella* serotypes. SERE-PCR identified five distinct fingerprint profiles among the 54 Enteritidis isolates; no correlation between phage types and SERE-PCR fingerprint patterns was noticed. SERE-PCR was reproducible, rapid and easy to perform. The results of this investigation suggest that the limited heterogeneity of SERE-PCR fingerprint patterns can be utilised to develop serotype- and serogroup-specific fingerprint patterns for isolates of Enteritidis.

Introduction

Several short, non-coding, inter-cistronic repetitive DNA sequences have been identified in prokaryotes [1], including the repetitive extragenic palindrome (REP) [2–4], enterobacterial repetitive intergenic consensus (ERIC) [5, 6] and BOX elements of *Streptococcus pneumoniae* [7]. The highly conserved nature and the widespread distribution of these repeat sequences in the bacterial genome have facilitated their use in the genetic fingerprinting of bacteria – for example, repetitive sequence-based polymerase chain reaction (rep-PCR), in which a DNA sequence, similar or identical to the repeat sequence, is used as primer to generate a specific pattern or genomic DNA fingerprint [1, 8, 9]. In previous studies, rep-PCR-generated fingerprints have been found to be species- or strain-specific and have proved useful for strain typing [8–11]. Because of its ease of use and discriminatory power, rep-PCR has become an important tool with which to fingerprint bacteria involved in outbreaks of disease and to determine the sources and vehicles of transmission [1, 10–12].

In this study, the existence of a novel, highly-

conserved repetitive DNA element located within the regulatory region of the *sefABCD* operon of the Enteritidis genome and its application in a PCR assay (SERE-PCR) for the genetic fingerprinting of isolates of Enteritidis and other serotypes of *Salmonella* from diverse O-serogroups are described.

Materials and methods

Bacterial strains

The properties of 54 isolates of *S. Enteritidis* (Table 1) and 54 isolates of other serotypes of *Salmonella* of diverse O-serogroups (Table 2) used in this study are shown. The sources of other bacterial species used in dot-blot hybridisation assays have been described previously [13]. Strains of *Salmonella* Typhi (2304), *Citrobacter diversus* (5403), *Enterobacter sakazakii* (4584) and *Str. pneumoniae* (424) were kindly provided by Dr E. O. Mason, Texas Childrens Hospital, Houston, TX, USA. Other strains were from the culture collections of the authors.

Isolation of chromosomal DNA

Bacteria were grown in Luria-Bertani (LB) broth, or were scraped directly from LB agar plates, and chromosomal DNA was extracted as described earlier [13].

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Corresponding author: Dr V. Kapur.

Table 1. Properties of 54 isolates of *Salmonella* serotype Enteritidis

Strain no.	Source, state (if USA) or country	Phage type	Fingerprint pattern
CA110	Chicken intestine, CA	1	B
M46	Chicken ovary, ME	2	C
M47	Environment, ME	2	C
M48	Environment, ME	2	C
Cal1*	Cat intestine, CA	4	B
Cal2	Chicken liver, CA	4	B
Cal3	Chicken liver, CA	4	B
Cal4	Mouse liver, CA	4	A
Cal5	Chicken ovary, CA	4	B
Cal6	Creek water, CA	4	B
Cal7	Skunk liver, CA	4	B
Cal8	Chicken egg, CA	4	B
Cal11	Creek water, CA	4	B
Cal12	Creek water, CA	4	B
Cal13	Creek water, CA	4	B
Cal14	Sewage, CA	RDNC	B
Cal15	Sewage, CA	RDNC	B
Cal16	Mouse liver, CA	4	B
Cal17	Sewage, CA	4	B
Cal9	Chicken liver, CA	7	B
M49	Chicken, Venezuela	7var	C
B7001	Chicken ovary, GA	8	C
M29	Chicken organ, ME	8	C
M470	Visceral organ, broiler, ME	8	C
CD14	Chicken, TX	8	C
N17	Turkey, CO	8	C
35054	Chicken, MN	8	C
4051	Chicken, MN	8	C
3842	Chicken, MN	8	C
M50	Cloaca of hen, ME	9b	C
N8	Duck, MD	9b	C
M23	Environment, ME	13	C
M25	Chicken, ME	13a	C
B6996	Human, GA	13a	C
CD2	Restaurant, NJ	13a	C
CD11	Chicken, NJ	13a	C
M28	Cat faeces, ME	13a	C
M33	Chicken gut, ME	13a	C
M34	Chicken visceral organ, ME	13a	C
M35	Chicken ovary, ME	13a	C
M36	Chicken visceral organ, ME	13a	C
M44	Cat faeces, ME	13a	C
M45	Egg belt, ME	13a	C
75-Li-De	Chicken liver, ME	14b	C
75EB3	Environment, ME	14b	C
M17	Visceral organ of hen, ME	14b	C
M39	Egg belt, ME	14b	C
M42	Egg follicle, ME	14b	C
M43	Environment, ME	14b	D
N4	Cat, MA	28	C
N9	Goose, MA	28	C
SE28	Chicken, MN	28	C
N2	Chicken, Spain	RDNC	E
M40	Chicken ovary, ME	NK	C

RDNC, reacts but does not conform to specific phage type; NK, not known.

*Isolates designated Cal1–Cal8 and Cal11–Cal17 are from an outbreak in a layer flock.

Oligonucleotide synthesis and PCR analysis

All synthetic oligonucleotide primers used in these studies were obtained from Integrated DNA Technologies Inc. (Ames, IA, USA) or the Nucleic Acids Core Facility (Dept of Molecular and Human Genetics, Baylor College of Medicine, TX, USA). Reagents and enzymes used for PCR were obtained from Perkin Elmer (Foster City, CA, USA). Amplification reactions were performed in 30- μ l volumes with primer 50 pmol, 5 mM MgCl₂ and genomic DNA 100 ng as a template in a DNA thermal cycler (Perkin Elmer, Cetus 480). Amplification reactions were performed twice to confirm assay reproducibility. Conditions for amplifica-

tion included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (94°C for 1 min), annealing (50°C for 1 min), extension (72°C for 2 min) and a final extension (72°C for 15 min). PCR products were analysed on an agarose gel 1.5% w/v, stained with ethidium bromide 0.5 mg/L and photographed under UV transillumination.

5' End-labelling of oligonucleotide probe

Oligonucleotide primer SERE (50 pmol) was end-labelled with 5 μ l of [γ ³²P]ATP (specific activity 4500 Ci/mmol; ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) and T4 polynucleotide kinase (New

Table 2. Serotypes of *Salmonella* examined

O-serogroup	Serotype
B	Abortusequi, Agona, Chester, Derby, Brandenburg, Heidelberg, Indiana, Newbrunswick, Saintpaul, Schwarzengrund, Typhimurium
C1	Bareilly, Braenderup, Livingstone, Montevideo, Oranienburg, Tennessee
C2	Blockley, Glostrup, Hadar, Newport
C3	Albany, Kentucky
D1	Berta, Dublin, Durban, Eastbourne, Gallinarum, Javiana, Moscow, Miami, Panama, Pullorum, Rostock
D2	Fresno
E1	Anatum, Westhampton
E2	Binza, Cambridge, Portsmouth, Rosenthal
E3	Thomasville
E4	Chittagong, Senftenberg
G1	Chameleon, Havana
G2	Putten, Worthington
I	Gaminara
J	Berlin
M	Pomana
N	Urbana
O	Alachua
Q	Champaign

England Biolabs, Beverly, MA, USA) 20 U at 37°C for 45 min and the reaction was stopped by adding 0.5 M EDTA (2 µl). Probe was purified from the unincorporated label by ultrafiltration through Microcon 3 filters, according to the manufacturer's instructions (Amicon Inc., Beverly, MA, USA).

DNA dot-blot hybridisation

A dot-blot membrane [13] containing chromosomal DNA from 44 bacterial and archaeal species from seven phyla [14], was prepared by blotting chromoso-

mal DNA from specific bacterial and archaeal species (Fig. 2). Chromosomal DNA (100 ng) was diluted in distilled water 300 µl, heated at 100°C for 5 min and spotted on to a hybridisation membrane. DNA was denatured *in situ* with 0.4 N NaOH, rinsed with 1 × SSC (0.15 M NaCl, 0.015 M trisodium citrate), dried by blotting with filter paper and baked at 80°C for 1 h. Hybridisation solution was prepared as described previously [13]. The membrane was prehybridised at 65°C for 2 h and hybridised at 65°C for 18 h with denatured radiolabelled probe. After hybridisation, the blot was washed twice with 2 × SSC at room

Fig. 1. a, PCR amplification of chromosomal DNA of Enteritidis with: SERE alone (lane 2); *sefA* reverse primer (5'TAGTTTTGATACTGCTGAACGTAG) alone (3); both SERE and *sefA* reverse primer (4). A 123-bp DNA ladder (lane 1) was used as mol. wt standard. **b**, secondary structure of the promoter region of the *sefABCD* operon, showing involvement of SERE sequence (nucleotide positions 9–27 in dashed box) and possible association with the promoter region. The –10 region, ATG f-Met start codon and the ribosome binding site (rbs) are underlined.

temperature for 5 min and once with 2 × SSC/SDS 0.02% w/v for 5 min. The blot was then exposed to Hyperfilm (Amersham Life Science Inc., Arlington Heights, IL, USA) with two intensifying screens at −80°C for 18–24 h.

Southern blot hybridisation

Chromosomal DNA (1 µg) from various bacterial and archaeal species was digested with combinations of restriction endonucleases: either *Bam* HI/*Eco* RI or *Bam* HI/*Eco* RI/*Hind* III or *Bam* HI/*Hind* III. The restriction endonucleases were used at 40 U/reaction and digestion was at 37°C for 12 h. Digested DNA samples were separated on an agarose gel 1% w/v, stained with ethidium bromide 0.5 mg/L and transferred to a Sure Blot Hybridisation membrane by capillary transfer at room temperature for 15 h. DNA on the membrane was prehybridised and then hybridised with end-labelled

oligonucleotide probe, and blots were developed as described above.

Results

A repetitive DNA sequence (SERE)

Single primer-based PCR with sequence 5'-GTGAG-TATATTAGCATCCGCA resulted in amplification of several distinct DNA fragments of 400–2500 bp from *Enteritidis* chromosomal DNA (Fig. 1a). One copy of this repeat sequence, designated *S. Enteritidis* repeat element (SERE), is located within the regulatory region (Fig. 1b) of the *Enteritidis* *sefABCD* operon [15, 16]. A subsequent computerised search of DNA sequence databases (GenBank release 91) identified several bacterial and archaeal species containing sequences showing high (> 70%) sequence similarity with SERE (Table 3).

Table 3. Alignment of repeat units from different bacterial and archaeal species

Species or serotype	Gene/locus	Accession number	Nucleotide sequence
S. Enteritidis	<i>sefA</i>	L03833	GTGAGTATATTAGCATCCGCA
<i>S. Typhimurium</i>	<i>phoE</i>	X03928	GTGAGTATc a Tc GCATC a GC A
<i>S. Typhi</i>	<i>phoE</i>	X74595	GTGAGTATc a Tc GCATC a GC A
<i>S. Pullorum</i>	<i>citC</i>	D10257	t TGAa T t T ATTA G t AT g C G C g
<i>Escherichia coli</i>	<i>apt-adk</i>	M38777	GTc t Ga A T A T T A t t A T C C G C A
<i>Haemophilus influenzae</i>	<i>hiu32784</i>	U32784	GTGAGTg a Ag TAc C Ag C C G C A
<i>Klebsiella pneumoniae</i>	<i>dha</i>	U30903	Gg a AGTg TATc AGC Ag c g GC A
<i>Neisseria gonorrhoeae</i>	<i>por</i>	X53824	GTc Aa a TATt a TAg a C AT C C c C A
<i>N. meningitidis</i>	<i>23rRNA</i>	X67300	Gc c AGT A a Ac Tg GCATC C G C A
<i>Pseudomonas aeruginosa</i>	<i>oprL</i>	Z50191	t TGA Gg ATAc g AGC AT C a GC A
<i>Proteus mirabilis</i>	<i>PRMFLI</i>	L07270	GTGA c TATt T TAc C t T C t. c C A
<i>Serratia marcescens</i>	<i>SMACHITA</i>	L38484	GTGc GTAc a G TAc g Aa c C G C A
<i>Vibrio cholerae</i>	<i>fur</i>	M85154	GTGA c g ATAc c AGCATC a t C A
<i>Xanthomonas campestris</i>	<i>avrB53-2</i>	X78781	GTGA c TAc c TGA G C g T C g GC A
<i>Campylobacter fetus</i>	<i>sapA2</i>	S76860	GTGA t T'g TAT T AGCATC a a C A
<i>C. jejuni</i>	<i>rpoB</i>	X77304	GTGAa a AT T a T Aa c AT C C c C A
<i>Actinobacillus pleuropneumoniae</i>	<i>hemolysin</i>	M65808	c c GAGc ATAT T At CATg C G a A
<i>Flavobacterium meningosepticum</i>	<i>FVBASPENDO</i>	L37784	GTGA t Tg T Aa T At CATC a GC g
<i>Mycoplasma gallisepticum</i>	<i>atp</i>	X64256	GTGAGTATg c T AGC Ag g C G g A
<i>M. genitalium</i>	<i>mgpA</i>	M31431	GTGA c T t TAT a A t CAT C t Ga A
<i>Shigella boydii</i>	<i>sbu14469</i>	U14469	a T G c GT t TAT a AG t AT g C G C A
<i>Arthrobacter</i> sp.	<i>fcABC</i>	M93187	t TGA G c Ag AT T At C Ag C C G C c
<i>Bacillus subtilis</i>	<i>mcp,tlpA</i>	L29189	t TGA t TAc a Ag a Aa CAT C C G C A
<i>Clostridium acetobutylicum</i>	<i>lyc</i>	M68865	GTGA GTATAT A a c AT C A c a C A
<i>Chlamydia psittaci</i>	<i>ompA</i>	X12647	GTGA c g c c AT T AGCATC C G C g
<i>Deinococcus radiodurans</i>	<i>DNApol</i>	L14581	Gc GA Gg Ag t T T c a CAT C C G C A
<i>Halobacterium halobium</i>	<i>ish26-1</i>	X04832	GTGA Gg AT c T T At t g T a C G C A
<i>Leptospira biflexa</i>	<i>trpEG</i>	M22468	GTGA GTAT T g c T AGC Ag g C G g A
<i>Lactobacillus lactis</i>	<i>23rRNA</i>	X68435	GTGA Ga AT c T T At C c a C C G t A
<i>Listeria monocytogenes</i>	<i>secA</i>	L32090	Gg a AGT Aa g T T c GCATC C a C A
<i>Methanobacterium thermoautotrophicum</i>	<i>MTU19364</i>	U19364	GTc AGTAT T c c T t GC Ag C C G C A
<i>Mycobacterium leprae</i>	<i>U00020</i>	U00020	GTGA GTATAT Ag g AGg Ac C C G C c
<i>Nostoc</i> sp.	<i>NOSFTZOOP</i>	L32796	GTGA t TATg a T At C Ac t C G C A
<i>Rhodobacter capsulatus</i>	<i>hupR1</i>	M63670	Gc Gc Ga Ag AT c AGCATC C G C g
<i>Rhizobium meliloti</i>	<i>fixF</i>	M18272	Ga GA Gg Ag c T T c GCATC a GC A
<i>Rhodobacter sphaeroides</i>	<i>nifU</i>	M86823	Gc Gg a T c g AT T AGCA C a C G C A
<i>Streptococcus agalactiae</i>	<i>cpsD</i>	L09116	t TGA t TATAT T At C AT C g a t A
<i>Staphylococcus aureus</i>	<i>STAORPHI</i>	L19300	GTc AGT t T Aa T AGa t T C t GC A
<i>Str. equi</i>	<i>emmS2W60</i>	U04620	c TGA GTg Tg T T Aa C Ac C a GC A
<i>Str. faecalis</i>	<i>ENEMSPDPS</i>	M38386	GTGA c T Aa AT c g Gc t T C C G C A
<i>Str. pneumoniae</i>	<i>SPENDA</i>	X54225	Ga GA GTg T c T T Aa C Ag a C G C A
<i>Str. pyogenes</i>	<i>STRMRY</i>	M58461	Ga GA GT t T t T T AGCATC a c a A
'Thermophilic bacteria'	<i>TBAACP</i>	D12512	GT t t GTAT t T T AGCAT t C G C A

Nucleotides not matching the SERE sequence are presented in lower case. The DNA sequences listed share >70% homology with SERE and had consensus, 5'GTGANTNNNTTNGCNCNGCA at 70% sequence homology.

The computerised database search revealed a SERE consensus sequence 5'-GTGANTNNNNTTNGCNCN-GCA (5'-GTGANTDTNNTANCATCNGCA for gram-negative and 5'-GNGAGTANNNTADCANCCGCA for gram-positive bacteria at 70% sequence homology) present in species belonging to at least 42 genera of eubacteria and two genera of archaea (Table 3). Examination of the SERE element suggests the presence of a relatively well-conserved promoter-like hexanucleotide consensus ($T_{80\%}A_{95\%}T_{45\%}A_{60\%}A_{50\%}T_{96\%}$) at nucleotide positions 6–12, and a highly conserved trinucleotide sequence 5'GCA at the 3' end of the sequence. The presence of a promoter-like sequence suggests that SERE may be present within the regulatory region of bacterial genes, as is present upstream of the *sefABCD* operon, and that it may influence gene expression.

Conservation of SERE among different bacterial genera

To assess the conservation of SERE among eubacteria and archaea, a dot-blot assay was developed with specific chromosomal DNA from eubacterial and archaeal species belonging to seven phyla (Fig. 2).

Results of that analysis suggest that SERE was widely distributed among different genera in both eubacterial and archaeal kingdoms. Furthermore, hybridisation of restriction endonuclease-digested chromosomal DNA from several selected species with radiolabelled SERE oligonucleotide probe supported the dot-blot results and showed that multiple dispersed copies of SERE are present in the genomes of many divergent bacterial phyla (data not shown). Moreover, single primer amplification with SERE of chromosomal DNA yielded results consistent with those from dot-blot hybridisation (Fig. 3). Thus, complex SERE-PCR amplification patterns were observed with many of the species that reacted strongly by dot-blot. Together, these results indicate that SERE is well conserved amongst several distinct genera within eubacterial and archaeal kingdoms.

Application of SERE for genetic fingerprinting of *Enteritidis* isolates

The results of SERE-PCR analysis showed that 54 *Enteritidis* isolates belonging to nine phage types could be assigned to five closely related SERE-PCR patterns, designated A–E (Fig. 4). Sixteen *Enteritidis* isolates

Fig. 2. Distribution of SERE amongst 44 different bacterial species assessed by dot-blot hybridisation. **A1**, *Rhodobacter sphaeroides*; **A2**, *S. Enteritidis*; **A3**, *Xanthomonas holcicola*; **A4**, *Bacillus subtilis*; **A5**, *Treponema pallidum*; **A6**, *Deinococcus radiophilus*; **B1**, *Rhizobium meliloti*; **B2**, *C. diversus*; **B3**, *Haemophilus influenzae*; **B4**, *Listeria monocytogenes*; **B5**, *T. vincenti*; **B6**, *Thermus thermophilus*; **C1**, *Neisseria gonorrhoeae*; **C2**, *Klebsiella pneumoniae*; **C3**, *Vibrio vulnificus*; **C4**, *Staphylococcus aureus*; **C5**, *Leptospira interrogans*; **C6**, *Herpetosiphon giganteus*; **D1**, *N. meningitidis*; **D2**, *Ent. sakazakii*; **D3**, *Myxococcus xanthus*; **D4**, *Str. pneumoniae*; **D5**, *Chlorobium vibrioforme*; **D6**, *Halobacterium halobium*; **E1**, *Sphaerotilus natans*; **E2**, *Serratia marcescens*; **E3**, *Arthrobacter luteus*; **E4**, *Str. pyogenes*; **E5**, *Bacteroides fragilis*; **E6**, sterile distilled water; **F1**, *Escherichia coli*; **F2**, *Yersinia enterocolitica*; **F3**, *Streptomyces albus* G; **F4**, *Str. agalactiae*; **F5**, *Fusobacterium nucleatum*; **G1**, *S. Typhimurium*; **G2**, *Proteus vulgaris*; **G3**, *Nocardia aerocolonigenes*; **G4**, *Caryophanon latum*; **G5**, *Flavobacterium meningosepticum*; **H1**, *S. Typhi*; **H2**, *Pseudomonas aeruginosa*; **H3**, *Nocardia otitidiscaviarum*; **H4**, *Mycoplasma pneumoniae*; **H5**, *Flavobacterium okeanoikoites*.

Fig. 3. SERE-PCR fingerprints of 44 eubacterial and archaeal species analysed by dot-blot hybridisation (in Fig. 2).

Fig. 4. SERE-PCR fingerprint patterns of Enteritidis isolates showing five fingerprint patterns: lane 1, A; 2, B; 3, D; 4, E; 5, C.

belonging to phage types 1, 4, 7 and RDNC were assigned to pattern B; 35 isolates belonging to phage types 7var, 8 9b, 13, 13a, 14b, 28 and NK were assigned to pattern C; and one isolate each of phage types 4, 14b and RDNC were assigned to patterns A, D and E, respectively (Table 1). The index of discrimination (D) [17] of SERE-PCR for these 54 Enteritidis isolates was 0.50, a value considerably less than that achieved with phage typing ($D = 0.86$).

SERE-PCR fingerprinting of other *Salmonella* serotypes

When a series of 54 strains of other serotypes of *Salmonella*, including 12 of other O-serogroup D serotypes, was analysed by SERE-PCR, unique fingerprint patterns were obtained for most of the serotypes examined (Fig. 5). The SERE-PCR fingerprint patterns of Enteritidis strains were distinct from those of other salmonellae, including those of other serogroup D serotypes, suggesting that SERE-PCR may be useful for genetic fingerprinting of serotypes of diverse *Salmonella* serogroups.

Discussion

A novel repetitive DNA sequence, SERE, identified on the chromosome of *Salmonella* serotype Enteritidis has been shown to be present in a wide range of species

from the eubacterial and archaeal kingdoms. The widespread distribution and presence of internal promoter-like sequences suggest that SERE may be present within the regulatory regions of genes, a hypothesis further supported by the finding that the SERE sequence is located as a tandem inverted repeat forming a stable stem loop (Fig. 1b) within the regulatory region of the *sefABCD* fimbrial operon of Enteritidis [15].

Whilst the exact mechanism by which these repeat sequences have dispersed in the genome and the reasons for their conservation are not clear, several roles have been suggested for their existence. For example, some repeat sequences such as the *Escherichia coli* REP element have the potential to form stem-loops, thereby regulating gene expression by differential translation within polycistronic operons and retroregulation by mRNA stabilisation [18–21]. REP sequences have also been found to bind DNA polymerase I and DNA gyrases and are believed to be involved in the folding of the nucleoid into independent super-coiled looped domains [18, 19, 22, 23]. Alternate hypotheses for the widespread conservation and dispersal of these elements include the suggestion that they may propagate themselves as selfish DNA by gene conversion through RNA intermediates [24], or that they serve as recombination sites [25], and frequent exchange of information by recombination at these sites may serve to retain these repeated elements with a high degree of sequence conservation. Although the results of this investigation do not exclude any of these hypotheses, the presence of SERE in the regulatory region of bacterial genes, as well as the presence of promoter-like elements and evidence of secondary structures, suggest that SERE may be involved in the regulation of gene expression by mediating DNA–protein interactions.

In general, species that reacted strongly in the dot-blot assay yielded complex SERE-PCR patterns and *vice versa* (Figs. 2 and 3). However, with isolates of *C. diversus*, *Ent. sakazakii*, *Pseudomonas aeruginosa* and *Nocardia otitidiscaviarum*, the strong intensity of signal observed by dot-blot was not reflected by a correspondingly increased complexity of SERE-PCR fingerprint patterns. Although the reasons for this discrepancy are not exactly known, these results may be due to a greater spacing of the repetitive element, very close opposition of the repetitive element, or to the same orientation of the repeat sequences to each other within the genome. By contrast, isolates of *Str. pneumoniae*, *Str. agalactiae*, *Str. pyogenes*, *Leptospira interrogans*, *Fusobacterium nucleatum*, *Chlorobium vibrioforme* and *Halobacterium halobium* gave relatively complex SERE-PCR fingerprint patterns, although they reacted weakly on dot-blot (Fig. 3). These results may partly reflect an altered GC content in these species.

Fig. 5. SERE-PCR fingerprint profiles of serotypes of *Salmonella*. **A**, O-serogroup D serotypes: lane 1, Enteritidis; 2, Javiana; 3, Moscow; 4, Miami; 5, Durban; 6, Pullorum; 7, Panama; 8, Gallinarum; 9, Dublin; **B**, 19 serotypes of other O-serogroups: lane 1, Enteritidis; 2, Livingstone; 3, Brandenburg; 4, Arizonae; 5, Braenderup; 6, Oranienburg; 7, Heidelberg; 8, Worthington; 9, Hadar; 10, Typhimurium; 11, Blockley; 12, Chester; 13, California; 14, Bareilly; 15, Abortusequi; 16, Anatum; 17, Tennessee; 18, Kentucky; 19, Drypool; 20, Senftenberg.

The results of this investigation showed SERE-PCR to be less discriminatory than phage typing, a finding that may suggest that resistance or susceptibility to bacteriophages is under strong evolutionary pressure and, hence, evolving at a more rapid rate than the rest of the bacterial genome. The five distinct SERE-PCR fingerprint patterns identified among isolates of Enteritidis were very similar. This limited heterogeneity of fingerprint patterns among Enteritidis isolates from various distinct host species and geographic locations may reflect either a relatively recent origin of serotype Enteritidis or a widespread dissemination of one highly virulent Enteritidis clone. These

hypotheses are consistent with the findings of a multilocus enzyme electrophoretic analysis of Enteritidis isolates that revealed a restricted genetic diversity in this serotype and showed that most (93%) Enteritidis isolates from world-wide sources were represented by one clone, En1 [26]. Analysis of Enteritidis strains from sporadic cases and outbreaks by pulsed field-gel electrophoresis and ribotyping have also indicated the limited genetic diversity of this serotype [27].

In conclusion, the results of the present investigation show that the paucity of heterogeneity in SERE-PCR

fingerprint patterns may be utilised to develop species- and serogroup-specific fingerprint patterns for isolates of *S. Enteritidis*. Further studies to determine whether the amplified fragments are unique to *S. Enteritidis* are in progress.

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