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Note

Phage-type specific markers identified by Diversity Arrays Technology (DArT) analysis of *Salmonella enterica* ssp. *enterica* serovars Enteritidis and Typhimurium

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ABSTRACT

Diversity Arrays Technology (DArT) was applied to differentiate between *S. enterica* serovar Enteritidis and Typhimurium strains, respectively. Ten and eleven, mainly phage and plasmid–related markers were identified for serovars Enteritidis and Typhimurium. In combination, these markers can be used for subtyping among and within phage types.

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Infections with *Salmonella enterica* subspecies 1 serovars Enteritidis and Typhimurium are among the most common causes of bacterial food-borne diseases in developed countries. In epidemiological investigations, differentiation of strains beyond serotyping is accomplished through traditional phage typing complemented by molecular typing methods. The classical, phenotypic phage typing method has several drawbacks including its empirical nature, which necessitates considerable routine and experience in classification, and the envisaged replenishment of phage stocks, which jeopardizes the consistency of the original phage-typing schemes (Schmieger, 1999). Among various molecular approaches to bacterial strain typing, pulsed field gel electrophoresis (PFGE) is considered as the "gold standard" technique (Lukinmaa et al., 2004), while high discriminatory power has also been displayed e.g. by amplified length polymorphism analysis (e.g. Lindstedt et al. 2000; Lan et al., 2007) or multilocus sequence typing (e.g. Ross and Heuzenroeder, 2005). In recent years, DNA microarray techniques have increasingly been applied for molecular strain identification, enabling highly multiplexed and high-throughput analyses (Pelludat et al., 2005; Wan et al., 2007). Here a novel approach to the microarray-based subtyping of *S. enterica* serovars Enteritidis and Typhimurium is presented, which is based on the Diversity Arrays Technology (DArT). The DArT platform screens a large number of genomic DNA fragments to identify sequence-ready diagnostic markers (Jaccoud et al., 2001; Kilian et al., 2005).

One hundred and seventy-nine strains of *S. enterica* were obtained from the Austrian Agency for Health and Food Safety (AGES) and the Veterinary Laboratories Agency (VLA) Weybridge. The strains comprised commonly found phage types (PT) 1, 4, 5, 6, 6a, 7, 8, 14b and 21 of serovar Enteritidis and "definite" phage types (DT) 1, 3, 12, 41, 46, 85, 104L, 104H, 120, 141, 193, 208, U291 and U302 of serovar Typhimurium (Table 1). Two separate DArT arrays were constructed by spotting individual DNA fragments from two serovar-specific genomic representations. The representations were generated by applying a "complexity-reduction method" (Wenzl et al., 2004) to a mixture of DNA preparations from all strains belonging to either serovar. The complexity-reduction method consisted in the amplification of short adapter-ligated restriction fragments. One hundred ng of each DNA mixture were digested and adapter-ligated in a 10 µl solution containing 2 units of both *Mse*I and *Eco*RI (Invitrogen), 1 × One-phor-all buffer (GE Healthcare Europe GmbH), 1 µg µl^{-1} BSA, 10 mM ATP, 1.2 Weiss Units T4 DNA ligase (Promega) and 1 pmol of each adapter. A standard adapter was ligated to the *Eco*RI ends (Vos et al., 1995). A long asymmetric adapter with a 3′ amino group on the short strand (5′-CCTG ATCGCTACAACTGAACGATGAGTCCTGAG-3′ annealed to 3′-NH$_2$-CTCA

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Table 1
Strains of *S. enterica* serovar Enteritidis (PT) and *S. enterica* serovar Typhimurium (DT) included in the study.

Phage type	Strain[a]	H[b]	Phage type	Strain	H	Phage type	Strain	H	Phage type	Strain	H
PT 1	06/733	■	PT 5	04/9207		PT 6a	S44-06		PT 14b	06/361	■
PT 1	06/754	■	PT 5	04/9343		PT 6a	S1290-06	■	PT 14b	06/393	■
PT 1	06/818 I	■	PT 5	S4543-04		PT 6a	S542-06	■	PT 14b	06/419	■
PT 1	06/831 II		PT 5	S4552-04	■	PT 7	05/5449	■	PT 14b	06/729	
PT 1	06/852		PT 5	S4554-04	■	PT 7	05/5537	■	PT 14b	06/753	
PT 1	S1831-06		PT 6	06/413	■	PT 7	05/6390	■	PT 14b	S630-05	
PT 1	S1750-06	■	PT 6	06/517	■	PT 7	06/415 II		PT 14b	S6199-05	■
PT 1	S3539-06	■	PT 6	06/637	■	PT 7	06/524		PT 14b	S544-06	■
PT 4	06/791	■	PT 6	06/688		PT 7	S2980-04		PT 21	06/812	■
PT 4	06/801	■	PT 6	06/822		PT 7	S3243-04	■	PT 21	06/820	■
PT 4	06/808	■	PT 6	S3693-06	■	PT 7	S3373-04	■	PT 21	06/851	■
PT 4	06/831 I		PT 6	S4280-06		PT 8	06/849	■	PT 21	06/867	
PT 4	06/860		PT 6	S4380-06		PT 8	06/859	■	PT 21	06/870	
PT 4	S672-06	■	PT 6	S4381-06	■	PT 8	06/862	■	PT 21	S172-05	
PT 4	S1094-06	■	PT 6a	05/7180	■	PT 8	06/863		PT 21	S964-05	■
PT 4	S1973-06		PT 6a	05/7475	■	PT 8	06/868		PT 21	S6564-03	■
PT 5	04/8071	■	PT 6a	05/7609	■	PT 8	S627-05				
PT 5	04/8461	■	PT 6a	06/254		PT 8	S4376-06	■			
PT 5	04/8855	■	PT 6a	06/555		PT 8	S7162-05	■			
DT 01	06/000063	■	DT 41	S2142-06	■	DT 104H	06/00000		DT 193	S3273-06	■
DT 01	06/000064	■	DT 46	05/000068	■	DT 104H	S5080-04		DT 193	S3327-06	■
DT 01	06/000069	■	DT 46	05/000072	■	DT 104H	S3168-05	■	DT 208	02/0000830	■
DT 01	06/000073	■	DT 46	05/000075	■	DT 104H	S2982-03	■	DT 208	03/0000703	■
DT 01	06/000004		DT 46	05/000077	■	DT 120	06/00000	■	DT 208	04/0000091	
DT 01	S1714-05	■	DT 46	06/000008	■	DT 120	06/00000	■	DT 208	04/0000191	
DT 03	04/000040	■	DT 85	05/000023	■	DT 120	06/00000	■	DT 208	04/0000589	
DT 03	05/000037	■	DT 85	05/000030	■	DT 120	06/00000		DT 208	S4956-06	
DT 03	05/000051	■	DT 85	05/000055	■	DT 120	06/00000		DT 208	S4959-06	
DT 03	05/000061	■	DT 85	05/000063		DT 120	S11-06		DT 208	S3828-04	■
DT 03	05/000077		DT 85	06/000003		DT 120	S2135-06		DT 208	S3747-04	■
DT 03	HPA	■	DT 85	S3823-05		DT 120	S1202-05		DT U291	04/0000643	■
DT 12	06/000081	■	DT 85	S5200-06	■	DT 120	S2473-06	■	DT U291	04/0000870	■
DT 12	06/000006	■	DT 85	S1046-03	■	DT 120	S2481-06	■	DT U291	04/0000907	■
DT 12	06/000056	■	DT 104L	06/000004	■	DT 141	00/9007	■	DT U291	04/0000930	■
DT 12	06/000059		DT 104L	06/000005	■	DT 141	04/00000	■	DT U291	06/0000052	■
DT 12	06/000066		DT 104L	06/000005	■	DT 141	04/00000	■	DT U302	03/0000105	■
DT 12	S51-06		DT 104L	06/000006		DT 141	04/00004	■	DT U302	05/0000246	■
DT 12	S1878-06	■	DT 104L	06/000008		DT 141	04/00005		DT U302	05/0000570	■
DT 12	S2186-06	■	DT 104L	S339-06		DT 141	S10325-	■	DT U302	05/0000666	
DT 41	05/000076	■	DT 104L	S1917-06		DT 193	05/00005	■	DT U302	05/0000734	
DT 41	06/000001	■	DT 104L	S2909-06	■	DT 193	05/00006	■	DT U302	S9-06	
DT 41	06/000004	■	DT 104L	S3437-06	■	DT 193	05/00006	■	DT U302	S4702-05	
DT 41	06/000005		DT 104H	06/000025	■	DT 193	05/00007		DT U302	S2878-04	■
DT 41	06/000007		DT 104H	06/000027	■	DT 193	05/00000		DT U302	S5619-03	■
DT 41	S1909-06	■	DT 104H	06/000038	■	DT 193	S60-06				
DT 41	S1963-06		DT 104H	06/000047		DT 193	S1045-06				

[a] Original laboratory identification.
[b] Strain used for hybridization.

GGACTCAT-5′) was ligated to the *Mse*I ends to prevent amplification of *Mse*I–*Mse*I fragments as a result of PCR suppression (e.g. van der Linden et al., 2004). Adapter-ligated fragments were amplified in 50 µl reactions containing 2 U of Taq polymerase (Invitrogen), 10 pmol of 5′-CCTGATCGCTACAACTGAAC-3′, 10 pmol of 5′-GACTGCGTACCAATTC-3′ and 1 µl of the digestion/ligation solutions as templates. PCR conditions were 94 °C (1 min), 30 cycles of 94 °C (20 s), 56 °C (40 s) and 72 °C (1 min), and a final extension at 72 °C for 7 min. Amplified fragments were ligated to the StrataClone pSC-A vector (Stratagene) and transformed into SoloPack PCR Cloning Competent Cells (Stratagene). Three thousand and seventy-two colonies of each representation were grown in 150 µl "LB freezing medium" (Wittenberg et al., 2005). 2-µl aliquots of overnight cultures were used as templates to amplify individual inserts with M13 primers flanking the multiple cloning site of the vector. Air-dried PCR products were dissolved in spotting buffer (3 × SSC, 1.5 M betaine, 0.01% SDS) and spotted in triplicates on poly L-lysine slides (Erie) using an OmniGrid arrayer (Biorobotics, UK). The slides were denaturated in water at 92 °C for 2 min and were then washed in 0.1 mM DDT and 0.1 mM EDTA.

Genomic DNA samples from individual strains were processed with the same complexity-reduction method to prepare target representations for array hybridizations, except that in the adapter and primer sequences G was exchanged for C and vice versa to prevent hybridization of adapter sequences (Wittenberg et al., 2005). Following Cy3-dCTP labeling and purification with the Bioprime Array CGH genomic labeling system (Invitrogen), 180 ng of individual target representations together with 9 µg salmon sperm DNA and 5 µl of Cy5-labeled vector DNA as a reference (Wittenberg et al., 2005) were added to 80 µl of Buffer Dig Easy (Roche), and following denaturation at 95 °C for 2 min were hybridized to the DArT arrays. After an overnight hybridization at 42 °C the arrays were washed (Kostic et al., 2005) and scanned using a Tecan LS Series Scanner. The images were analyzed with DArTsoft 7.2.9 (DArT Pty Ltd) to identify polymorphic markers and convert hybridization intensities into presence/absence scores. Each target was assayed (hybridized to microarrays) at least twice.

Sets of four to five strains, each representing a given phage type, were selected for analysis. Hybridization of genomic representations prepared from these strains with probes on the Enteritidis and Typhimurium DArT arrays allowed the rapid retrieval of sequences present in the genomes of some strains but absent from others. Probes were identified that produced contrasting scores for different phage types or for various strains within phage types. Eleven and 19 non-

redundant probes that discriminated among phage types or differentiated among groups of strains within phage types were identified for serovar Enteritidis and Typhimurium strains, respectively. On the Enteritidis array two probes were identified that were exclusively present in representations of the PT 5 strains. Because these two probes scored identically, a set of ten distinctive probes was used in the final set of diagnostic markers for characterizing serovar Enteritidis strains (Fig. 1). These ten markers, in combination, reported a single score pattern for the PT 5 strains, two different patterns for the PT 6 strains, three patterns for the PT 6a strains and two patterns for the PT 8 strains. In addition, groupings of strains representing more than one phage type were formed based on the combined scores of these markers. For instance, three of the PT 8 strains grouped together with one of the PT 14b strains, while the two other PT 8 strains analyzed gave a unique pattern (Fig. 1). Out of the 19 discriminative probes obtained from the Typhimurium array, 11 were selected as markers because their combined scorings provided phage-type relevant information (Fig. 2).

Phage type	Strain	DArT markers										Pattern
		32G24	31N21	32N14/32D4	28F6	31J22	30J1	33F24	29A6	30M14	29B7	
PT1	S1750-06	1	0	0	1	0	1	0	0	0	1	■
PT1	S3539-06	1	0	0	1	0	1	0	0	0	1	■
PT1	06/733	1	0	0	1	0	1	0	0	0	1	■
PT1	06/818I	1	0	0	1	0	1	0	0	0	1	■
PT1	06/754	1	0	0	1	0	0	0	0	0	1	○
PT4	S672-06	1	0	0	1	0	0	0	0	0	1	○
PT4	S1094-06	1	0	0	1	0	0	0	0	0	1	○
PT4	06/791	1	0	0	1	0	0	0	0	0	1	○
PT4	06/801	1	0	0	1	0	0	0	0	0	1	○
PT4	06/808	1	0	0	1	0	0	0	0	0	1	○
PT5	S4552-04	0	0	1	1	0	0	0	0	0	1	▲
PT5	S4554-04	0	0	1	1	0	0	0	0	0	1	▲
PT5	04/8071	0	0	1	1	0	0	0	0	0	1	▲
PT5	04/8461	0	0	1	1	0	0	0	0	0	1	▲
PT5	04/8855	0	0	1	1	0	0	0	0	0	1	▲
PT6	06/413	1	0	0	1	0	0	0	0	0	1	○
PT6	06/517	1	0	0	1	0	0	0	0	0	1	○
PT6	06/637	1	0	0	1	0	0	0	0	0	1	○
PT6	S3693-06	1	1	0	1	0	0	0	0	0	1	▼
PT6	S4381-06	1	1	0	1	0	0	0	0	0	1	▼
PT6a	05/7180	1	0	0	1	1	1	0	1	0	0	◀
PT6a	05/7475	1	0	0	1	1	1	0	1	0	0	◀
PT6a	05/7609	1	0	0	1	1	1	0	0	0	0	◀
PT6a	S1290-06	1	0	0	1	0	1	1	0	0	1	□
PT6a	S542-06	1	0	0	1	0	1	1	0	0	1	□
PT7	S3243-04	1	0	0	1	0	0	0	0	0	1	○
PT7	S3373-04	1	0	0	1	0	0	0	0	0	1	○
PT7	05/5449	1	0	0	1	0	0	0	0	0	1	○
PT7	05/6390	1	0	0	1	0	0	0	0	0	1	○
PT7	05/5537	1	0	0	1	0	1	0	0	0	1	■
PT8	06/849	0	0	0	1	0	0	0	0	1	1	◆
PT8	06/859	0	0	0	1	0	0	0	0	1	1	◆
PT8	06/862	0	0	0	1	0	0	0	0	1	1	◆
PT8	S4376-06	0	1	0	0	0	0	0	0	1	1	–
PT8	S7162-05	0	1	0	0	0	0	0	0	1	1	–
PT14b	06/419	1	0	0	1	0	0	0	0	0	1	○
PT14b	06/393	1	0	0	1	0	1	1	0	0	1	□
PT14b	S6199-05	1	0	0	1	0	1	1	0	0	1	□
PT14b	S544-06	1	0	0	1	0	1	1	0	0	1	□
PT14b	06/361	0	0	0	1	0	0	0	0	1	1	◆
PT21	S964-05	1	0	0	1	0	1	0	0	0	1	■
PT21	S6564-03	1	0	0	1	0	1	0	0	0	1	■
PT21	06/812	1	0	0	1	0	1	0	0	0	1	■
PT21	06/820	1	0	0	1	0	1	0	0	0	1	■
PT21	06/851	1	0	0	1	0	1	0	0	0	1	■

Fig. 1. DArT markers of *S. enterica* serovar Enteritidis that give phage-type relevant 0/1 (absent/present) scoring patterns. Distinct scoring patterns are marked with specific symbols that are also shown in Fig. 3.

Phage type	Strain	DArT markers											Pattern
		23A18	23I17	18C18	25O21	23O2	24C4	24G7	22L1	24K3	18J13	25D3	
DT1	06/00006348	0	0	0	0	0	0	0	1	0	1	1	
DT1	06/00006961	0	0	0	0	0	0	0	1	0	1	1	
DT1	06/00007368	0	0	0	0	0	0	0	1	0	1	1	
DT1	S1714-05	0	0	0	0	0	0	0	1	0	1	1	
DT1	06/00006465	1	1	0	0	1	0	0	0	0	0	0	
DT3	HPA control	1	0	1	0	1	1	1	1	0	0	0	
DT3	04/00004076	1	0	1	0	1	1	0	1	1	1	0	○
DT3	05/00003757	1	0	1	0	1	1	0	1	1	1	0	○
DT3	05/00005115	1	0	1	0	1	1	0	1	1	1	0	○
DT3	05/00006192	1	0	1	0	1	1	0	1	1	1	0	○
DT12	06/00008190	1	1	1	0	1	1	1	1	0	1	0	
DT12	06/00005663	1	1	1	0	1	1	0	1	0	1	0	
DT12	S1878-06	1	1	1	0	1	1	0	1	1	1	0	
DT12	S2186-06	1	1	1	0	1	1	0	1	1	1	0	
DT12	06/00000667	1	1	1	0	1	0	0	1	0	0	0	■
DT41	06/00000195	0	0	0	0	0	0	0	0	0	1	0	
DT41	06/00000441	0	0	0	0	0	0	0	0	0	1	0	
DT41	S1909-06	1	0	0	0	1	1	1	1	0	1	0	
DT41	S2142-06	1	0	0	0	1	1	1	1	0	1	0	
DT41	05/00007627	1	0	0	0	1	1	1	1	0	1	0	
DT46	05/00006811	1	0	1	0	1	1	0	1	0	1	0	◆
DT46	05/00007292	1	0	1	0	1	1	0	1	0	1	0	◆
DT46	05/00007595	1	0	1	0	1	1	0	1	0	1	0	◆
DT46	05/00007709	1	0	1	0	1	1	0	1	0	1	0	◆
DT46	06/00000827	1	0	1	0	1	1	0	1	0	1	0	◆
DT85	05/00002386	1	0	1	0	1	1	0	1	0	1	0	◆
DT85	05/00003003	1	0	1	0	1	1	0	1	0	1	0	◆
DT85	S5200-06	1	0	1	0	1	1	0	1	0	1	0	◆
DT85	S1046-03	1	0	1	0	1	1	0	1	0	1	0	◆
DT85	05/00005565	1	0	1	0	1	1	0	1	0	1	0	◆
DT104H	S3168-05	1	1	0	0	0	0	0	1	0	1	1	□
DT104H	S2982-03	1	1	0	0	0	0	0	1	0	1	1	□
DT104H	06/00002548	1	1	0	0	0	0	0	1	0	1	1	□
DT104H	06/00002710	1	1	0	0	0	0	0	1	0	1	1	□
DT104H	06/00003844	1	1	0	0	0	0	0	1	0	1	1	□
DT104L	S2909-06	1	1	0	0	0	0	0	1	0	1	1	□
DT104L	S3437-06	1	1	0	0	0	0	0	1	0	1	1	□
DT104L	06/00000443	1	1	0	0	0	0	0	1	0	1	1	□
DT104L	06/00000536	1	1	0	0	0	0	0	1	0	1	1	□
DT104L	06/00000578	1	1	0	0	0	0	0	1	0	1	1	□
DT120	06/00000647	0	0	0	0	1	0	0	1	0	1	1	
DT120	06/00000601	1	1	0	0	1	1	0	1	1	0	0	
DT120	S2473-06	1	1	0	0	0	0	0	1	0	0	1	
DT120	S2481-06	1	1	0	0	0	0	0	1	0	0	1	
DT120	06/00000799	1	1	0	0	0	0	0	1	0	1	1	□
DT141	S10325-90	1	0	0	0	1	1	0	1	0	1	0	
DT141	00/9007	1	0	0	0	1	1	0	1	0	1	0	
DT141	04/00000179	1	0	0	0	1	1	0	1	0	1	0	
DT141	04/00000645	1	0	0	0	1	1	0	1	0	1	0	
DT141	04/00004814	1	0	0	0	1	1	0	1	0	1	0	
DT193	S3273-06	0	0	0	1	1	0	0	1	1	0	0	
DT193	S3327-06	0	0	0	1	1	0	0	1	1	0	0	
DT193	05/00006347	1	0	0	0	1	1	0	1	1	1	0	
DT193	05/00006364	1	0	1	0	1	1	0	1	1	1	0	○
DT193	05/00005563	1	1	1	1	1	1	0	1	1	0	0	
DT208	S3828-04	1	1	1	0	1	0	0	1	0	0	0	■
DT208	S3747-04	1	1	1	0	1	0	0	1	0	0	0	■
DT208	02/00008303	1	1	1	0	1	0	0	1	0	0	0	■
DT208	03/00007032	1	1	1	0	1	0	0	1	0	0	0	■
DTU291	04/00006432	1	0	0	0	1	1	1	0	0	1	0	
DTU291	04/00008706	1	0	0	0	1	1	1	0	0	1	0	
DTU291	04/00009071	1	0	0	0	1	1	1	0	0	1	0	
DTU291	04/00009306	1	0	0	0	1	1	1	0	0	1	0	
DTU291	06/00000521	1	0	0	0	1	1	1	0	0	1	0	
DTU302	S2878-04	0	0	0	0	1	0	0	1	0	1	0	
DTU302	05/00005701	0	0	0	0	1	0	0	0	0	0	1	
DTU302	S5619-03	1	0	0	0	0	0	0	0	0	1	0	
DTU302	03/00001054	1	1	0	0	0	0	0	1	0	1	1	□
DTU302	05/00002467	1	1	0	0	0	0	0	1	0	1	1	□

Fig. 2. DArT markers of *S. enterica* serovar Typhimurium that give phage-type relevant 0/1 (absent/present) scoring patterns. Scoring patterns that are common to two or several phage types are marked with a specific symbol for each pattern. Patterns that are unique for a single phage type have no symbol.

The specific scoring patterns reported by these markers allowed full discrimination of DT 1, DT 141, DT 41 and DT U291 strains. In addition, DT 46 and DT 85 strains together showed distinctive scores, and DT 104H and DT 104L strains together with one of the DT 120 and two of the DT U302 strains produced a unique scoring pattern (Fig. 2).

S. enterica serovar Enteritidis strains that were hybridized onto the DArT array were also analyzed via PFGE as described in Beranek et al. (2009). Relatedness among the PFGE profiles was evaluated with Bionumerics software (version 3.0; Applied Maths, Ghent, Belgium), revealing 5 major lineages which were essentially similar to the major lineages identified through the DArT application (Fig. 3). As with the DArT method, only the PT 5 strains could be fully resolved in the PFGE analysis based on a threshold of ≥90% similarity; and a similar relationship was apparent between the PT 8 and one of the PT 14b strains by both methods. The discriminatory power of the DArT application presented here, however, may still be improved through increased multiplexing. Advantages of a microarray-based method over PFGE include that it is high-throughput and easier to standardize. DArT provides sequence-ready markers and thus allows insights into the molecular differences between *Salmonella* phage types.

Sequencing of diagnostic markers (amplified plasmid inserts) was done with the ABI Prism Big Dye terminator cycle sequencing kit (PE

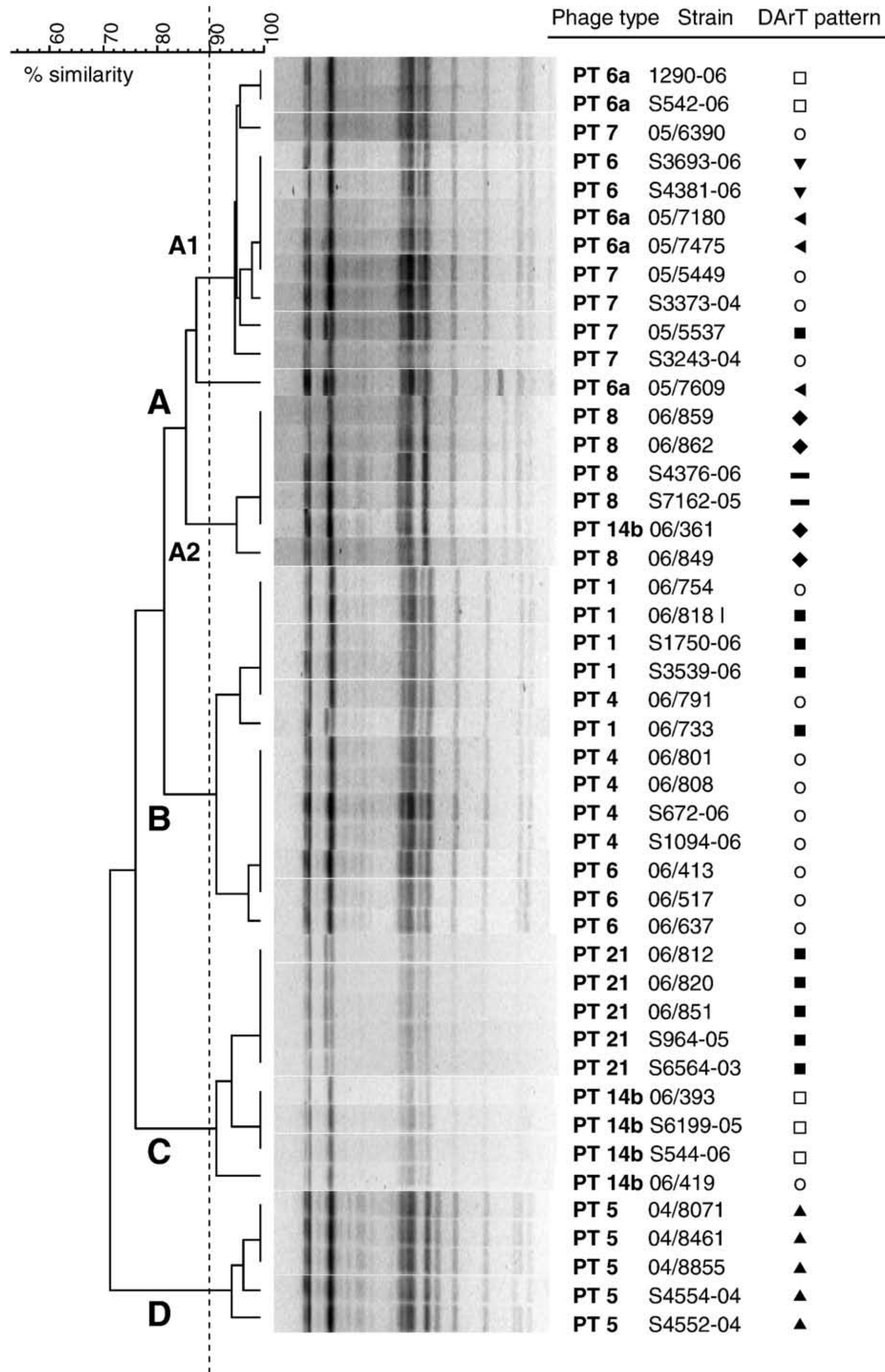

Fig. 3. Dendrogram (based on an unweighted pair group method using arithmetic averages (UPGMA) and use of the Dice coefficient) of the PFGE data of the 45 *S. enterica* serovar Enteritidis strains that were analyzed via the DArT-based method. The strains are divided into five major clusters with one strain showing a unique banding pattern based on a threshold of ≥90% similarity. Phage types, strains and respective DArT patterns (see Fig. 1) are plotted next to the dendrogram.

Table 2
Results of tBlastX-based sequence analysis done on DArT markers for *S. enterica* serovar Enteritidis and Typhimurium strains shown in Figs. 1 and 2.

Marker	Identity (%)	Accession number	NCBI entry	Function
Serovar Enteritidis				
32G24	100	AM933172	putative tail protein [*Salmonella typhimurium* phage ST64B]	(unknown)
31N21	99	ZP_00695472	ATPase involved in DNA repair [*Shigella boydii* BS512]	MobA/MobL family, DNA repair
32N14	100	AF479774	PsiB-like hypothetical protein [*Klebsiella pneumoniae* plasmid pJHCMW1]	(establishment after conjugation)
32D4	98	AAL21505	hypothetical protein STM2610 [phage Gifsy-1 organism = *Salmonella typhimurium* LT2]	(similar to *E. coli* orf, hypothetical protein (AAC74262.1))
28F6	100	NP_458090	phosphate transport system permease protein [*S. enterica* serovar Typhi str. CT18]	Inorganic ion transport and metabolism
31J22	100	AAZ05390	TraF protein [*S. enterica* plasmid pNF1358]	Transfer protein
30J1	100	YP_218607	putative transcriptional regulator [*Salmonella enterica* serovar Choleraesuis str. SC-B67]	Helix lactose operon repressor
33F24	100	AAC27625	methyl-accepting chemotaxis-like protein [*Pseudomonas aeruginosa* RP-1, plasmid pPa-1]	Chemotaxis sensory transducer
29A6	100	AAZ05393	type III pepilin [*S. enterica* plasmid pNF1358]	Peptidase, bacterial shufflon protein
30M14	99	AAL21609	terminase-like protein [phage Fels-2, organism = *Salmonella typhimurium* LT2]	Packaging viral DNA into the capsid
29B7	100	AAV78815	Orn/Lys/Arg decarboxylase, ornithine decarboxylase isozyme [*S. enterica* ser. Paratyphi A str. ATCC 9150]	Amino acid transport and metabolism, role in acid tolerance
Serovar Typhimurium				
23A18	85	NP_700396	putative tail protein Sb23 [*S. typhimurium* phage ST64B]	(unknown)
23I17	47	NP_455519	putative prophage membrane protein DUF754 [*S. enterica* subsp. enterica serovar Typhi str. CT18]	(unknown)
18C18	94	YP_217620	Gifsy-1 prophage cI protein [*Salmonella enterica* serovar Choleraesuis str. SC-B67]	Role in lysis-lysogeny decision in bacteriophage lambda and related phages
25O21	32	XP_847514	MutL, mismatch repair protein MLH1 [*Trypanosoma brucei* TREU927]	predicted ATPase
23O2	100	YP_151404	glyoxylate carboligase [*Salmonella enterica* serovar Paratyphi A str. ATCC 9150]	(general function prediction only)
24C4	100	NP_700375	Terminase large subunit Sb2 [*Salmonella typhimurium* phage ST64B]	(general function prediction only)
24G7	98	NP_958059	putative capsid scaffold gp4 [Bacteriophage PSP3; *Pseudomonas* phage PsP3]	Assembly of proheads and cleavage of GPN
22L1	100	AAL21530	STM2636, Gifsy-1 prophage protein [*S. typhim.* LT2]	Bacteriophage P4 integrase
24K3	98	YP_151802	putative phage gene [*S. enterica* serovar Paratyphi A str. ATCC 9150]	Dam, site-specific DNA methylase
18J13	97	NP_490495	*repC* DNA replication protein [*Salmonella typhimurium* LT2, plasmid = pSLT]	*repC* DNA replication protein
25D3	98	NP_461546	hypothetical protein STM2610 [Phage Gifsy-1]	Bacterial pre-peptidase C-terminal domain

Applied Biosystems Inc.), and sequences were blasted against the National Center for Biotechnology Information (NCBI) database (Altschul et al., 1997). As observed in other molecular approaches to *Salmonella* subtyping (e.g. Hu et al., 2002; Ross and Heuzenroeder, 2005), the majority of diagnostic marker sequences were homologues to sequences of phage or plasmid origin, e.g. ST 64B, Fels-2 and Gifsy-1 phages or *S. enterica* plasmids pNF1358 and pSLT (Table 2), which account for most of the genetic diversity among closely related *Salmonella* strains (Figueroa-Bossi and Bossi, 2004). Mobile genetic elements such as plasmid or phage genes have been implicated in the horizontal transfer of virulence genes (Boyd and Brussow, 2002), and phage-type conversion through plasmid or phage acquisition has been reported (e.g. Mmolawa et al., 2002). Thus, the DArT markers allude to epidemiological characteristics and may add to our understanding of the emergence and evolution of *S. enterica* subtypes. Moreover, they may help to elucidate the molecular basis of the classical typing scheme, since expression of prophage genes may mediate resistance of the host bacterium to superinfecting phages and thereby influence its phenotypic phage type (Tucker and Heuzenroeder, 2004).

The DArT platform used in the present study rapidly identified sequence-ready markers that distinguished among *S. enterica* phage types. For proof-of-concept purposes a complexity-reduction method was selected that generated genomic representations with a limited number of DNA fragments. Experience with DArT genotyping of other organisms, however, has shown that the size of genomic representations could easily be increased by one to two orders of magnitude (Kilian et al., 2005). Such an increased multiplexing level may retrieve a sufficient number of markers to differentiate among yet unresolved phage types and to subtype highly clonal strains within phage types. We suggest that the appropriate DArT marker sets, once established for the most prevalent phage types of *S. enterica* serovars Enteritidis and Typhimurium, be arrayed on diagnostic biochips to serve as time and cost efficient, highly parallel and high-throughput subtyping tools in epidemiological investigations.

The nucleotide sequences of the DArT markers were deposited with GenBank under accession numbers FJ884197 to FJ884218.

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