Multiple-locus variable-number tandem repeat analysis for discrimination of *Salmonella enterica* strains belonging to different serovars

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Summary

Seven variable-number tandem repeat (VNTR) markers, which had previously proven to be suitable for typing of *Salmonella enterica* serovar Typhimurium or *S. enterica* serovar Enteritidis, were evaluated for their discrimination power in multilocus VNTR analysis (MLVA) on a panel of 83 *S. enterica* strains belonging to 23 different serovars, which had been isolated from food and animal samples. Strains varied not only in numbers of tandem repeats, but also in the presence or absence of VNTR alleles. The greatest polymorphism was observed in STTR5, STTR3 and STTR7 loci. Based on combined allelic profiles of all seven VNTR loci, it was possible to distinguish as many as 50 different MLVA profiles. The MLVA clustering of strains parallelled serotyping results to a reasonable extent. In addition, a high degree of intra-serovar variability was observed in strains of serovars Bareilly, Bredeney, Dublin, Enteritidis, Havana, Saintpaul, Senftenberg, Schwarzengrund and Typhimurium. MLVA proved to be more discriminative than *dna*N housekeeping gene sequencing. The optimized MLVA method can be advantageously used in tracing of *Salmonella* contamination of food products.

Keywords

Salmonella; genotyping; tandem repeat; food borne pathogens; DNA; PCR

Food-borne infections caused by *Salmonella enterica* represent a major healthcare concern worldwide, including the most developed countries. According to the current taxonomy, genus *Salmonella* is separated into two species and six subspecies containing more than 2500 different serovars. Although all of them are considered as potential human pathogens, the majority of human infections are caused by a limited number of serovars of *S. enterica* subsp. *enterica* [1].

Subspecies typing of isolated strains represents a crucial step in early detection and control of outbreaks. Serotyping of the flagellar and O-polysaccharide antigens is the most commonly used approach to characterization of *Salmonella* strains. For discrimination of strains within serovars, numerous DNA-based methods were established, including plasmid profiling, ribotyping, pulsed field gel electrophoresis (PFGE), repetitive element-primed PCR (REP-PCR) and amplified fragment length polymorphism (AFLP) [2–4]. In recent years, the advance in DNA sequencing techniques facilitated the introduction of promising new typing methods such as multilocus sequence typing (MLST) and multiple-locus variable-number tandem repeat analysis (MLVA).

MLST is based on the comparison of nucleotide sequences of several housekeeping genes distributed throughout the bacterial genome. The MLST protocol for *Salmonella* is based on seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*). The protocol was evaluated [5] and the international database of alleles and strain types became available [6]. Although MLST is a robust technique, its discriminatory power is only moderate as the variability between strains of the same serovar is limited.

MLVA is a technique which detects variations in the copy number of direct repeats (VNTR stands for variable number of tandem repeats) between strains. Although repetitive DNA occurs frequently in eukaryotic genomes, the repetitive

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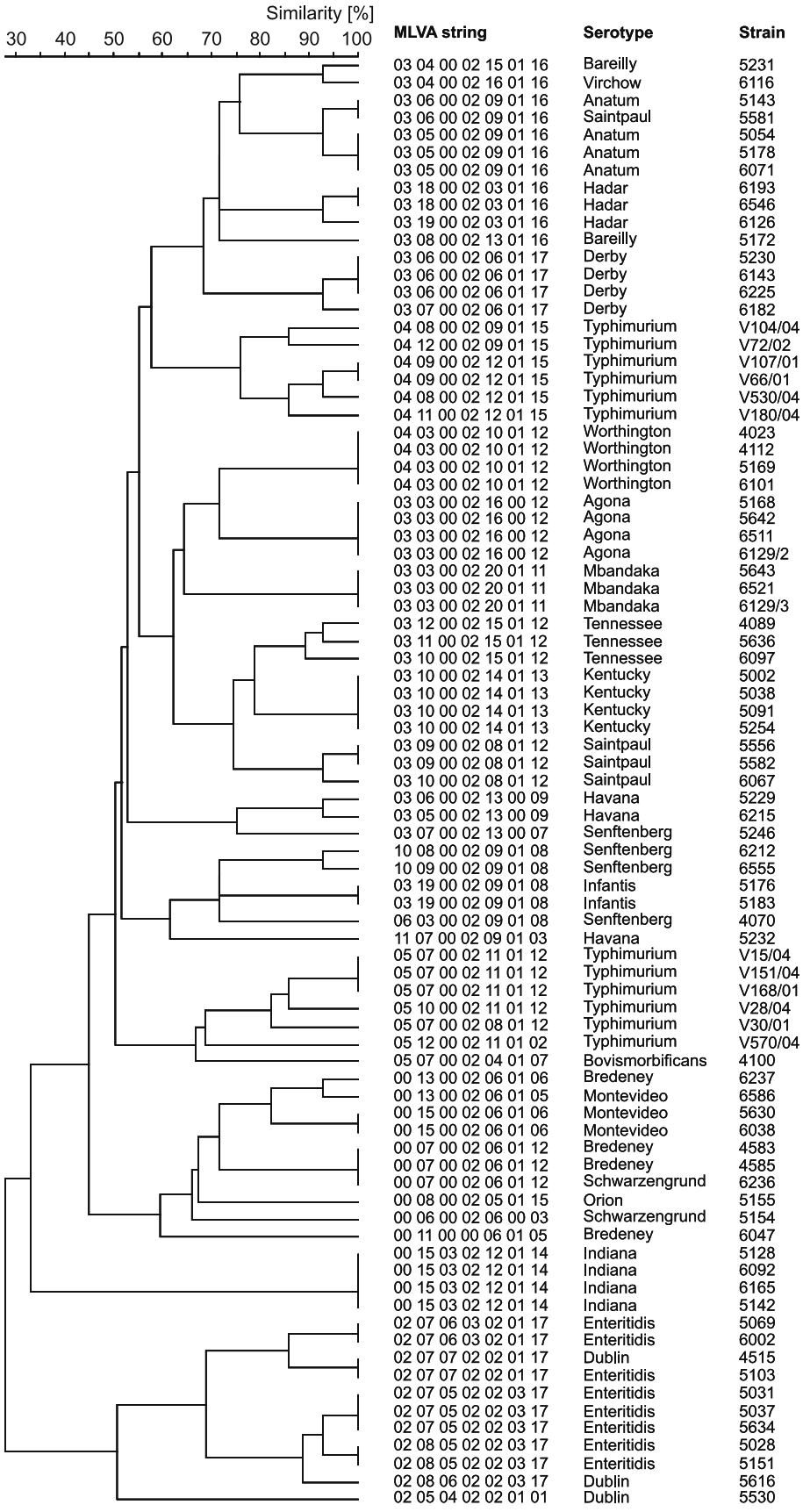


Fig. 1. Dendrogram of 83 *Salmonella enterica* strains based on MLVA typing.
Allele strings are expressed in the order of increasing molecular weight: STTR9-STTR5-SE1-SE9-STTR3-SE8-STTR7.

DNA stretches in prokaryotic genomes are relatively seldom and were detected mainly by whole-genome scanning. The underlying mechanism of VNTR is believed to be the slipped-strand mispairing coupled with imperfect DNA mismatch repair during replication. Estimated frequency of mutation event is 10^{-4} per cell division. Some VNTR loci are localized inside genes and their copy number variation may help to adapt the encoded protein to the environmental selective pressure. The high VNTR heterogeneity among bacterial strains was used for sensitive DNA fingerprinting. However, an individual MLVA scheme must be developed for each particular taxonomic group, e. g. *Haemophilus influenzae*, *Bacillus anthracis* or *Mycobacterium tuberculosis* [7, 8], because the presence and variability of VNTR loci is specific for individual species and/or serovars. For salmonellae, MLVA schemes were published which proved its usefulness particularly for typing of frequently occurring taxons, e. g. *S. typhi* [9], *S. enterica* serovar Typhimurium [10, 11] and *S. enterica* serovar Enteritidis [12–14]. The variability of ten VNTR loci between strains of several serovars of *S. enterica* subsp. *enterica* was studied by Rammisse et al. [15], who found that the most variable were strains of *S. typhi*, while other *Salmonella enterica* serovars showed only a low level of intra-serovar polymorphism.

The aim of our study was gain new knowledge on the polymorphism of tandem repeat loci in various *Salmonella enterica* serovars, and to assess the discriminatory power of these markers in *Salmonella enterica* typing.

MATERIALS AND METHODS

Bacterial strains

A number of 83 *Salmonella* strains used in this study were isolated during 2001–2006 from food and animal samples at the State Veterinary and Food Institute, Bratislava, Slovakia as part of a surveillance programme. Strains belonged to 23 serovars (Fig. 1). Strains were routinely cultivated aerobically in liquid or solid Luria-Bertani medium at 37 °C overnight.

MLVA typing

Chromosomal DNA was isolated by chaotropic solid-phase extraction using the DNeasy kit (Qiagen, Hilden, Germany).

For typing of *Salmonella* strains, seven VNTR loci were used according to previously published reports (Tab. 1). All loci were amplified in a single multiplex PCR containing 60 nmol·l^{-1} STTR3, 20 nmol·l^{-1} STTR5, 40 nmol·l^{-1} STTR7, 24 nmol·l^{-1} STTR9, 32 nmol·l^{-1} SE1, 40 nmol·l^{-1}

Tab. 1. Primers used in the study.

Locus	Primer designation	Sequence	Reference
STTR3	STTR3F-Joe	CCC CCT AAG CCC GAT AAT GG	[10]
	STTR3R	TGA CGC CGT TGC TGA AGG TAA TAA	
STTR5	STTR5F-Joe	ATG GCG AGG CGA GCA GCA GT	[10]
	STTR5R	GGT CAG GCC GAA TAG CAG GAT	
STTR7	STTR7F-Fl	CGC GCA GCC GTT CTC ACT	[10]
	STTR7R	TGT TCC AGC GCA AAG GTA TCT A	
STTR9	STTR9F-Fl	AGA GGC GCT GCG ATT GAC GAT A	[10]
	STTR9R	CAT TTT CCA CAG CGG CAG TTT TTC	
SE1	Se1F-Tamra	AGA CGT GGC AAG GAA CAG TAG	[12]
	Se1R	CCA GCC ATC CAT ACC AAG AC	
SE8	Se8F-Tamra	TTG CCG CAT AGC AGC AGA AGT	[12]
	Se8R	GCC TGA ACA CGC TTT TTA ATA GG	
SE9	Se9F-Fl	CGT AGC CAA TCA GAT TCA TCC C	[12]
	Se9-R	TTT GAA ACG GGG TGT GGC GCT G	
dnaN	DnaNF-PCR	ATG AAA TTT ACC GTT GAA CGT GA	[5]
	DnaNR-PCR	AAT TTC TCA TTC GAG AGG ATT GC	
	DnaNF-seq	CCG ATT CTC GGT AAC CTG CT	
	DnaNR-seq	CCA TCC ACC AGC TTC GAG GT	

SE8 and 16 nmol·l^{-1} SE9 primers, 200 μmol·l^{-1} of each dNTP, 1.5 U Go Hot Start Taq DNA polymerase (Promega, Madison, Wisconsin, USA), 1× PCR buffer and 4 μg·l^{-1} (2 μl of the stock solution) template DNA in a 25 μl reaction volume. The thermocycler programme consisted of the initial denaturation for 10 min at 95 °C, 30 cycles of amplification (30 s at 94 °C, 90 s at 58 °C, 90 s at 72 °C), and a final polymerization for 30 min at 60 °C. Fluorescently labelled PCR products were separated by capillary electrophoresis on the ABI Prism 3100 Avant DNA analyzer (Applied Biosystems, Foster City, California, USA) with ILS-600 size standard (Promega). Electrophoretograms were evaluated by using GeneMapper software (Applied Biosystems) and the allele numbers were assigned for particular VNTR loci based on the repeat unit count. The dendrogram of strain relatedness was constructed based on allelic profiles of all loci using BioNumerics software (AppliedMaths, Sint-Martens-Latem, Belgium). Mutual similarity between strains was calculated using the multistage categorical coefficient with a tolerance level of 2 and a fuzzy logic option. Unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm was employed for dendrogram construction.

Sequencing of *dna*N gene

An amount of 80 nmol·l^{-1} of primers DnaNF-PCR and DnaNR-PCR (Tab. 1) were used for amplification in the reaction mixture containing 200 μmol·l^{-1} of each dNTP, 1.5 U Go Taq DNA polymerase , 1× PCR buffer and 4 μg·l^{-1} of the template DNA in a total volume of 25 μl. PCR products were purified by polyethyleneglycol precipitation and sequenced using Big Dye Terminator 3.1 sequencing kit (Applied Biosystems) in both directions with primers DnaNF-seq and DnaNR-seq. Sequencing products were separated on ABI Prism 3130xl genetic analyzer (Applied Biosystems), sequences were evaluated and clustered using BioNumerics software.

RESULTS AND DISCUSSION

Multilocus VNTR analysis of *Salmonella* strains

The VNTR polymorphism has been described as an efficient typing method for various bacterial species. Several highly discriminative protocols were developed for analysis of *S. typhi*, *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium [9–15]. In our previous work, MLVA protocol based on five loci (STTR3, STTR5, STTR6, STTR9 and STTR10) [11] was successfully used for typing of *S. enterica* serovar Typhimurium strains originating from food, human and veterinary infections [16]. In the present study, we aimed to adapt this protocol further for discrimination of other *S. enterica* serovars. However, we recognized that STTR6 and STTR10 loci, although highly discriminative in *S. enterica* serovar Typhimurium, were not amplified in strains of other *S. enterica* serovars and thus the discriminative power of the method was substantially reduced (data not shown). Consequently, according to preliminary experiments (data not shown), we selected a new set of VNTR loci to evaluate their potential in typing of a broader panel of *S. enterica* serovars; four loci (STTR3, STTR5, STTR7 and STTR9) originally described for *S. enterica* serovar Typhimurium [10] and three loci (Se1, Se8 and Se9) previously used for analysis of *S. enterica* serovar Enteritidis [12].

The protocol for MLVA was optimized. Each of VNTR regions was amplified with fluorescently labelled primers flanking the repeated sequences and PCR products were separated by capillary electrophoresis (Fig. 2). DNA fragments were sized based on ILS-600 DNA standard and they were assigned to the allele numbers for particular VNTR loci according to the repeat count. The results of MLVA typing for each strain were expressed as allele strings in the order of increasing molecular weight: STTR9-STTR5-SE1-SE9-STTR3-SE8-STTR7 (Fig. 1).

The optimized MLVA was evaluated on a panel of 83 *Salmonella* strains of 23 serovars frequently occurring in Slovak Republic. The VNTR loci differed in numbers of amplified alleles and in the number of strains not amplifying the particular VNTR locus (Tab. 2, Fig. 1). The allele distribution of particular VNTR loci differed in serovars (Tab. 3). Four STTR loci originally used for *S. enterica* serovar Typhimurium subtyping showed a higher polymorphism than the SE loci used in *S. enterica* serovar Enteritidis typing scheme.

STTR3 locus was present in all *S. enterica* strains and fifteen different alleles were detected. Besides 33 bp differences between STTR3 alleles corresponding to the size of the repetition unit, alleles with other size difference were observed, containing incomplete repetitions. The length of PCR product was mainly serovar-specific, intraserovar polymorphism was present in serovars Bareilly, Havana, Saintpaul, Senftenberg and Typhimurium.

STTR5 locus containing the 6 bp repetition unit was amplified in all strains. Fifteen different alleles were detected in our strain collection and STTR5 was the most polymorphic marker. This

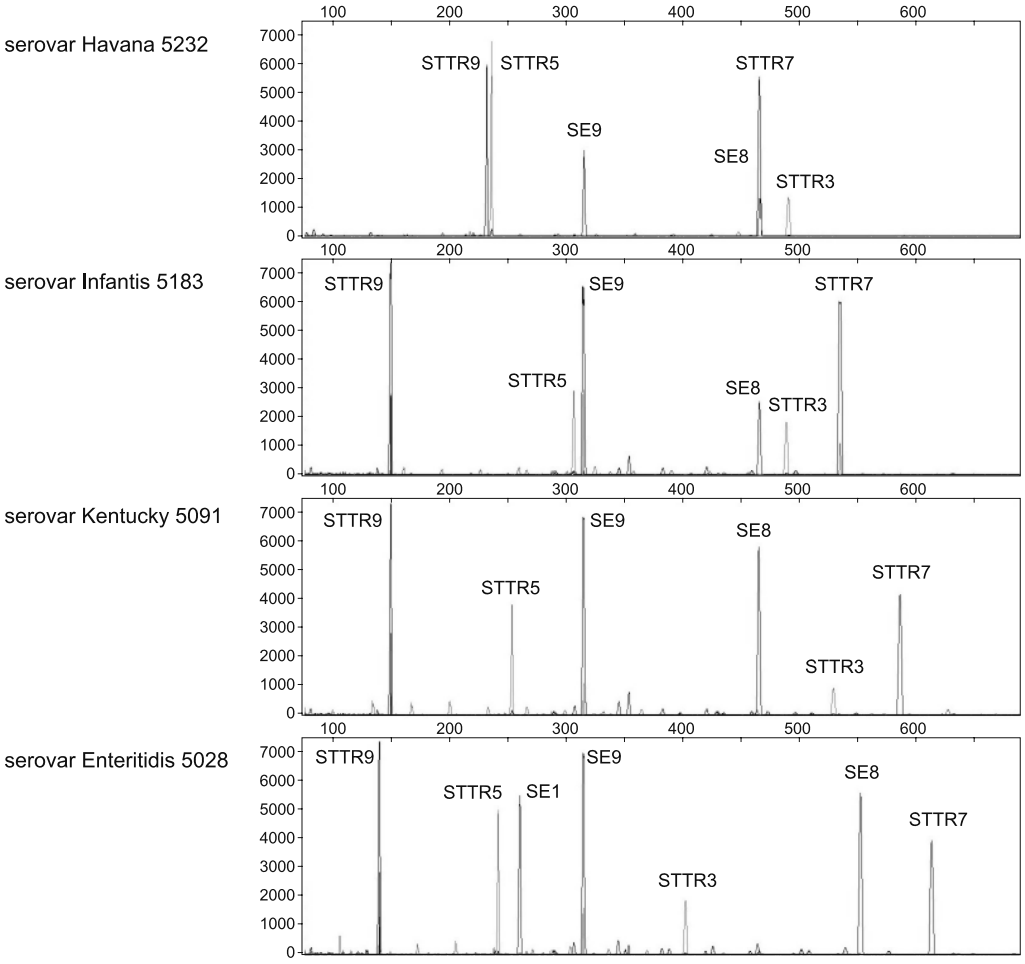


Fig. 2. Electrophoretograms of VNTR PCR products of *Salmonella enterica* strains.

locus showed also the greatest intra-serovar variability, it was the only polymorphic marker for serovars Anatum, Derby, Hadar and Tennessee. This locus appeared to be the most valuable for discrimination of *S. enterica* strains by VNTR polymorphism. It is therefore not surprising, that it was used (under various names) in several published *Salmonella* MLVA typing schemes [10–15]. STTR5 is a part of *yoh*M gene encoding for nickel/cobalt efflux protein [17], but the biological significance of its length variability remains unclear.

Similarly, STTR7 locus was present in all *S. enterica* strains and fifteen different alleles were detected. Quite variable length distribution was observed, alleles were mostly serovar-specific. Intra-serovar polymorphism was present in

Tab. 2. VNTR loci used for typing of *Salmonella* strains.

Locus	Gene	Repetitive region size [bp]	Smallest product [bp]	Largest product [bp]	Number of alleles	Missing allele [%]	Simpson's index of diversity
STTR9	intergenic	9	139	231	7	16,9	0.742
STTR5	*yoh*M	6	205	306	15	0	0.909
SE1	intergenic	7	246	273	5	81,9	0.325
SE9	*ush*A	9	314	323	2	1,2	0.071
STTR3	*big*A	variable (33)	403	732	15	0	0.913
SE8	*pip*B2	87	466	553	2	9,6	0.298
STTR7	*fts*K	variable (39)	307	616	15	0	0.874

Tab. 3. MLVA variability in *Salmonella enterica* serovars.

Serovar	Number of strains	Number of MLVA types	Polymorphic loci
Agona	4	1	
Anatum	4	2-SLV	STTR5
Bareilly	2	2	STTR5, STTR3
Bovismorbificans	1	1	
Bredeney	4	3	STTR5, Se9, STTR7
Derby	4	2-SLV	STTR5
Dublin	3	3	STTR5, Se1, Se8, STTR7
Enteritidis	8	4	STTR5, Se1, Se9, Se8
Hadar	3	2-SLV	STTR5
Havana	3	3	STTR9, STTR5, STTR3, Se8, STTR7
Indiana	4	1	
Infantis	2	1	
Kentucky	4	1	
Mbandaka	3	1	
Montevideo	3	2	STTR5, STTR7
Orion	1	1	
Saintpaul	4	3	STTR5, STTR3, STTR7
Senftenberg	2	2	STTR5, STTR3, Se8, STTR7
Schwarzengrund	2	2	STTR5, Se8, STTR7
Tennessee	3	3-SLV	STTR5
Typhimurium	12	9	STTR9, STTR5, STTR3, STTR7
Virchow	1	1	
Worthington	4	1	

SLV – single locus variants, differences in only one locus were detected.

serovars Bredeney, Dublin, Havana, Montevideo, Saintpaul, Senftenberg, Schwarzengrund and Typhimurium.

Seven different alleles of the STTR9 locus were observed in the analysed strains. The locus was not amplified in 14 strains belonging to serovars Bredeney, Indiana, Montevideo, Orion and Schwarzengrund. Intra-serovar polymorphism was present in *S. enterica* serovar Havana and *S. enterica* serovar Typhimurium. The allele No. 3 was the most frequent with 45% occurrence.

The SE loci, which were originally developed for *S. enterica* serovar Enteritidis MLVA, showed little variability in strains other than *S. enterica* serovar Enteritidis and *S. enterica* serovar Dublin. The SE1 locus was amplified in only three serovars – Indiana, Dublin and Enteritidis, and it was polymorphic only in strains of the latter two serovars. Four alleles were present and Simpson's index of diversity reached 0.64 in these serovars. The SE8 and SE9 loci were the least polymorphic ones in our set, with only two size variants in *S. enterica* serovar Enteritidis and *S. enterica* serovar Dublin strains.

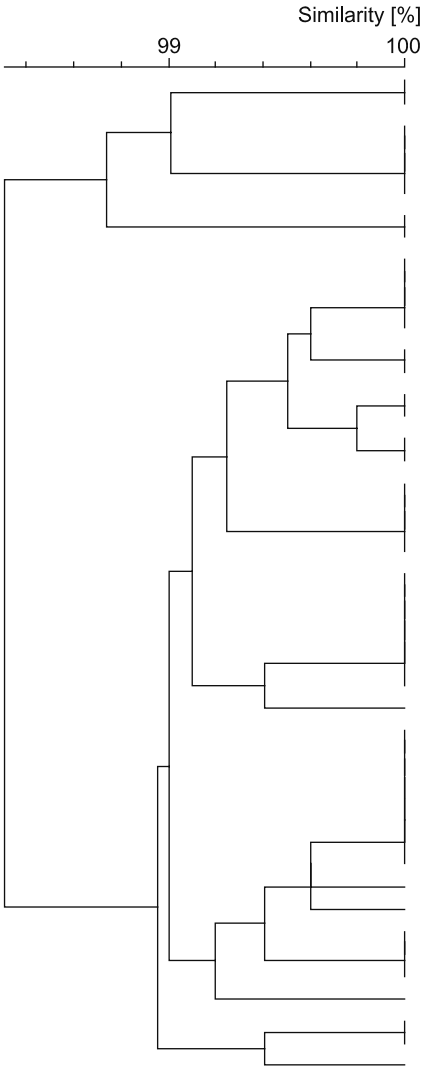
Based on the allelic profiles of all seven VNTR loci, mutual similarity between *Salmonella* strains and the dendrogram of strain relatedness was calculated using the multistage categorical coefficient and UPGMA clustering algorithm by BioNumerics software (Fig. 1). The number of 83 *S. enterica* strains were resolved into 50 different MLVA profiles. Strains were clustered mainly according to the serovars. Strains of *S. enterica* serovar Enteritidis and *S. enterica* serovar Dublin were separated to a common independent cluster with only 30% similarity to other serovars. The serovar-specific alleles in the STTR3, STTR7 and STTR9 loci as well as polymorphisms in SE loci were responsible for this clustering. Similarly, four strains of *S. enterica* serovar Indiana with identical MLVA profile showed low similarity to other strains. In other *S. enterica* serovars, the MLVA profiles were not separated to clear serovar-specific clusters.

The intra-serovar variability highly varied between serovars (Tab. 3). Six serovars did not show any inter-serovar length polymorphism. Only minor variations limited to the STTR5 locus were observed in serovars Anatum, Derby, Hadar and Tennessee. The highest variability was present in serovars Bareilly, Havana, Saintpaul, Senftenberg and Typhimurium, where substantial length polymorphisms in several loci were observed and consequently strains of the same serovar were localized in distant areas of the dendrogram. It is possible that these strains belong to several clonal lineages. It is however important to note, that due

to scarce availability of strains of some serovars, the observed intra-serovar variability might have been affected by differences in numbers of analysed strains of different serovars.

Sequencing of *dna*N gene

In the second part of our study, variability of MLVA was compared to variability of DNA sequences of a housekeeping gene. We sequenced the *dna*N gene coding for DNA polymerase III beta subunit, which is one of seven housekeeping loci used in MLST typing protocol for *S. enterica* [6]. Two strains from one serovar showing the greatest possible dissimilarity in MLVA profiles were selected for sequencing analysis; overall 45 representative strains of 20 serovars were used. We observed high conservation in the 501 bp internal fragment of *dna*N in the analysed strains, the sequence similarity reaching 98.3% (Fig. 3). Seventeen sequence variants were obtained, alleles were numbered according to the *Salmonella* MLST database [6]. Dominantly, no sequence differences were present in strains of the same serovar and, in a few cases, the identical allele was detected in two or three different serovars, e. g. serovars Anatum, Kentucky and Saintpaul showed a common allele 14, or serovars Enteritidis and Dublin had the allele 2. Intra-serovar variability of *dna*N sequence was detected in strains of serovars Bareilly, Havana and Senftenberg, which is in agreement with their great intra-serovar polymorphism in MLVA. Overall, Simpson's index of diversity in *dna*N sequences reached 0.936, which is much lower than 0.991 obtained for the same strain collection using the MLVA method.



*dna*N allele	Serotype	Strain
107	Tennessee	4089
107	Tennessee	6097
47	Bredeney	4583
47	Bredeney	6047
47	Schwarzengrund	5154
47	Schwarzengrund	6236
8	Indiana	5128
8	Indiana	6092
7	Typhimurium	V15/04
7	Typhimurium	V30/01
7	Typhimurium	V570/04
7	Typhimurium	V66/01
18	Infantis	5176
18	Infantis	5183
20	Derby	5230
20	Derby	6182
3	Agona	5168
3	Agona	5642
70	Mbandaka	5643
70	Mbandaka	6129/3
70	Worthington	4023
70	Worthington	4112
14	Anatum	5054
14	Anatum	5143
14	Kentucky	5002
14	Kentucky	5038
14	Saintpaul	5556
14	Saintpaul	5581
47	Schwarzengrund	5154
47	Schwarzengrund	6236
6	Senftenberg	6212
2	Dublin	4515
2	Dublin	5530
2	Enteritidis	5028
2	Enteritidis	5031
2	Enteritidis	6002
193	Havana	5229
174	Havana	5232
5	Bareilly	5172
5	Hadar	6126
5	Hadar	6193
65	Senftenberg	5246
41	Montevideo	5630
41	Montevideo	6586
69	Bareilly	5231

Fig. 3. Dendrogram of 45 *Salmonella enterica* strains based on the *dna*N sequence.

CONCLUSION

The multilocus analysis of variable tandem repeats is a method which has a great potential in typing of *S. enterica* because of its sensitivity, reproducibility and speed. The method may be advantageously used in epidemiological investigations and in tracing of *Salmonella* contamination of food. Our study has shown that VNTR loci, although successful for typing of *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis, have a limited typing potential in serovars Agona, Anatum, Derby, Hadar, Indiana, Infantis, Kentucky, Mbandaka, Tennessee and Worthington, even when used in combination. On the other hand, the combined approach facilitated high discrimination of strains for serovars Bareilly, Bredeney, Dublin, Havana, Saintpaul, Senftenberg and Schwarzengrund.

Acknowledgements

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REFERENCES

1. Brenner, F. W. – Villar, R. G – Angulo, F. J. – Tauxe, R. – Swaminathan, B.: *Salmonella* nomenclature. Journal of Clinical Microbiology, *38*, 2000, pp. 2465–2467.
2. Torpdahl, M. – Skov, M. N. – Sandvang, D. – Baggesen, D. L.: Genotypic characterization of *Salmonella* by multilocus sequence typing, pulsed-field gel electrophoresis and amplified fragment length polymorphism. Journal of Microbiological Methods, *63*, 2005, pp. 173–184.
3. Lindstedt, B. A. – Heir, E. – Vardund, T. – Kapperud, G.: Fluorescent amplified-fragment length polymorphism genotyping of *Salmonella enterica* subsp. *enterica* serovars and comparison with pulsed-field gel electrophoresis typing. Journal of Clinical Microbiology, *38*, 2000, pp. 1623–1627.
4. Kuchta, T. – Drahovská, H. – Pangallo, D. – Siekel, P.: Application of polymerase chain reaction to food analysis. Bratislava : Food Research Institute, 2006. 108 pp. ISBN 80-969608-5-7.
5. Kidgell, C. – Reichard, U. – Wain, J. – Linz, B. – Torpdahl, M. – Dougan, G. – Achtman, M.: *Salmonella typhi*, the causative agent of typhoid fever, is approximately 50,000 years old. Infection Genetics and Evolution, *2*, 2002, pp. 39–45.
6. *Salmonella enterica* MLST Database. In: MLST Databases at the Environmental Research Institute, University College Cork [online]. Cork : University College Cork, sine dato [cit. 9 September 2009] <http://mlst.ucc.ie/mlst/dbs/Senterica>
7. Lindstedt, B. A.: Multiple-locus variable number tandem repeats analysis for genetic fingerprinting of pathogenic bacteria. Electrophoresis, *26*, 2005, pp. 2567–2582.
8. van Belkum, A.: Tracing isolates of bacterial species by multilocus variable number of tandem repeat analysis (MLVA). FEMS Immunology and Medical Microbiology, *49*, 2007, pp. 22–27.
9. Liu, Y. – Lee, M. A. – Ooi, E. E. – Mavis, Y. – Tan, A. L. – Quek, H. H.: Molecular typing of *Salmonella enterica* serovar Typhi isolates from various countries in Asia by a multiplex PCR assay on variable-number tandem repeats. Journal of Clinical Microbiology, *41*, 2003, pp. 4388–4394.
10. Lindstedt, B. A. – Heir, E. – Gjernes, E. – Kapperud, G.: DNA fingerprinting of *Salmonella enterica* subsp. *enterica* serovar Typhimurium with emphasis on phage type DT 104 based on variable number of tandem repeat loci. Journal of Clinical Microbiology, *41*, 2003, pp. 1469–1479.
11. Lindstedt, B.A. – Vardund, T. – Aas, L. – Kapperud, G.: Multiple-locus variable-number tandem-repeats analysis of *Salmonella enterica* subsp. serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. Journal of Microbiological Methods, *59*, 2004, pp. 163–172.
12. Boxrud, D. – Pederson-Gulrud, K. – Wotton, J. – Medus, C. – Lyszkowicz, E. – Besses, J. – Bartkus, J. M.: Comparison of multiple-locus variable number tandem repeat analysis, pulsed-field gel electrophoresis, and phage typing for subtype analysis of *Salmonella enterica* serotype Enteritidis. Journal of Clinical Microbiology, *45*, 2006, pp. 536–543.
13. Cho, S. – Boxrud, D. – Bartkus, J. – Whittam, T. S. – Saeed, A. M.: Multiple-locus variable-number tandem repeat analysis of *Salmonella* Enteritidis isolates from human and non-human sources using a single multiplex PCR. FEMS Microbiology Letters, *275*, 2007, pp. 16–23.
14. Beranek, A. – Mikula, C. – Rabold, P. – Arnhold, D. – Berghold, C. – Lederer, I. – Allerberger, F. – Kornschober, C.: Multiple-locus variable-number tandem repeat analysis for subtyping of *Salmonella enterica* subsp. *enterica* serovar Enteritidis. International Journal of Medical Microbiology, *299*, 2009, pp. 43–51.
15. Ramisse, V. – Houssu, P. – Hernandez, E. – Denoeud, F. – Hilaire, V. – Lisanti, O. – Ramisse, F. – Cavallo, J. D. – Vergnaud, G.: Variable number of tandem repeats in *Salmonella enterica* subsp. *enterica* for typing purposes. Journal of Clinical Microbiology, *42*, 2004, pp. 5722–5730.
16. Drahovská, H. – Mikasová E. – Szemes T. – Ficek A. – Sásik M. – Majtán V. – Turňa J.: Variability in occurrence of multiple prophage genes in *Salmonella* Typhimurium strains isolated in Slovak Republic. FEMS Microbiology Letters, *270*, 2007, pp. 237–244.
17. Thorgersen, M. P. – Downs, D. M.: Cobalt Targets multiple metabolic processes in *Salmonella enterica*. Journal of Bacteriology, *189*, 2007, pp. 7774–7781.

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