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Expression profiles of effector proteins SopB, SopD1, SopE1, and AvrA differ with systemic, enteric, and epidemic strains of *Salmonella enterica*

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The presence and expression of *sopB*, *sopD1*, *sopE1*, and *avrA* genes encoding virulence associated effector proteins were studied comparatively in 405 *Salmonella enterica* strains. They belong to different serovars and clonal types (genotypes, phage types) and originated from different clinical (systemic infection, focal enteritis, enterocolitis) and epidemic sources (epidemics, sporadic cases). The *sopB* and *sopD1* determinants were commonly prevalent, but *sopE1* and *avrA* genes only in 55% and 80%, respectively. A correlation of this pattern of absence and presence of the respective genes to the epidemic and clinical origin could not be detected. In contrast, the expression of the respective genes appeared differently: SopB and SopE1 proteins are well produced, but SopD1 and AvrA proteins only rarely under the applied standard culture conditions. However, using a range of different environmental signals (temperature, pH, cations, *etc.*) some of the *S. enterica* nonproducer strains (*e.g.*, *S.* Agona, *S.* Bovismorbificans, *S.* Virchow, *etc.*) begin to produce AvrA and SopD1. They turned now into an expression profile which was found typically for the epidemic strains of *S.* Typhimurium and *S.* Enteritidis. Also *S. enterica* strains from systemic infections could be characterized by their strong SopB and SopE1 expression while SopD1 and AvrA proteins were missing. Although it is premature to outline generally a correlation of these expression profiles and the clinical and epidemiological potency of Salmonellae, the reported results allow a first understanding how a fine tuning of their virulence will take place.

Keywords: Effector proteins / *Salmonella enterica* / Virulence

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1 Introduction

Salmonella enterica is one of the most common causes of food-borne infections worldwide [1, 2] affecting foods of animal as well as of plant origins (eggs, meat, sausages, sprouts, paprika powder, aniseed, *etc.*; see [3]). Although *S. enterica* appears with a broad range of genetically different serovars [4] and clonal types [5, 6], only a limited number of *Salmonella* variants have attracted clinical and epidemiological attention. For instance, *S. enterica* serovar Enteritidis PT4 has been identified as the most frequently occurring *Salmonella* strain in countries of the northern hemisphere [7], covering, *e.g.*, in Germany 60–80% of all cases of salmonellosis in the last ten years [8]. Moreover, various *Salmonella* strains (serovars) display mainly systemic disease, other strains mainly focal intestinal diseases (enteritis, enterocolitis). For instance, serovar *S.* Typhi remains restricted to systemic infections (typhoid fever) of humans, whereas other serovars, *e.g.*, *S.* Typhimurium, occur mainly with focal, self-limiting enteritis in humans and young animals [9].

A large number of virulence determinants of *S. enterica* strains responsible for this broad spectrum of pathogenicity have been identified in the past [10]. There is strong evidence that type III secretion systems [11] are involved in the virulence of salmonellae which can deliver an array of virulence-associated proteins (effector proteins) directly into the host cells though circumventing the uneconomic release of large amounts of virulence products into environmental habitats [12, 13]. A large number of such translocated effector proteins affecting various cellular functions have been identified [14–21]. For example, the effector protein SopB has been shown to dephosphorylate the second messenger molecule 1,2,3,4,5, pentakis-inositol phosphate [22] which interferes with the regulative network controlling the influx/efflux of water and electrolytes, the IL8 production (inflammation), as well as actin rearrangements [14, 22–30].

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Therefore, it was anticipated that differences in the presence and in the expression of genes encoding effector proteins (a likely candidate is *sopB*) might be the clue to identify the molecular background of systemic and focal enteric infections [14, 26, 31, 32]. However, by identifying the presence or absence of the various genes for effector proteins, it was shown that all *S. enterica* strains appeared rather conserved with respect to the incidence of their respective genes [31, 33 – 37]. As shown for *S*. Paratyphi B [34], a kind of strain-specific expression profile of some effector proteins might be responsible for differences in their virulence potency.

In order to study the differences among *S. enterica* strains with systemic or with focal intestinal clinical outcomes (both in their sporadic or epidemic appearance), epidemic and clinical isolates as well as reference strains were tested. PCR and Southern blots were applied to prove the presence of the virulence associated effector protein genes *sopB*, *sopE1*, *sopD1*, and *avrA* and Western blots to test their expression. These genes were chosen out of others because they are suggested to be involved either in the systemic (invasion) in the case of *sopE1* and *sopB,* or in the enteric (diarrhoea) pathogenesis in the case of *sopB* and *sopD1* [31]. Since the AvrA and SopD1 proteins were found to be rarely expressed among the strains under investigation, several environmental signals, such as temperature, pH, iron deficiency, *etc.*, were tested whether they could induce the effective expression.

2 Material and methods

2.1 Bacterial strains

Seventy-nine *S. enterica* strains of the SARB collection [5] and 326 respective epidemical and clinical isolates from human infections were chosen from the type culture collection of the National Reference Center of Salmonellae and other enterics (NRC), Wernigerode. Among them, various phage types and genotypes of *S. enterica* serovar Enteritidis, *S*. Typhimurium, and *S*. Paratyphi B (including the systemic and entric pathovars as described earlier [34]) were also chosen. As positive and negative controls, respectively, the isogenic reference strains *S*. Typhimurium SL1344 and SL1344-230 were applied. SL1344-230 carries a deletion in the translocon gene *invA* rendering it unable to translocate any effector protein into the culture supernatants (Hardt, personal communication). All strains had been stored as glycerol (20%) cultures at –70°C.

2.2 Plasmids and DNA techniques

The plasmids pETSopB-1344, pETSopD1-1344, and pETAvrA-1344 were applied for the production of respective proteins. They were derived using the pET-Directional TOPO® Expression Kit, Invitrogen BV (Breda, The Netherlands). All DNA techniques, such as PCR, Southern blots, transformation, and plasmid identifications, *etc.*, were carried out according to [33].

2.3 Culture conditions for the production of effector proteins

One colony of each respective *S. enterica* strains was resuspended in 5 mL Luria-Bertani broth (LB; Difco, Detroit, MI, USA) + 0.3 M NaCl and incubated for 6 h at 37°C (OD of 0.7 – 0.8) under shaking on a longitudinal shaker (100/min). 5 mL of this preculture was diluted into 15 mL fresh warm LB broth + 0.3 M NaCl using a 100 mL bulb flask with narrow neck and incubated over night on a longitudinal shaker (100 rpm). Variants of this standard culture conditions referred to temperature (30°C instead of 37°C), to pH 6.0 and pH 8.0 instead of pH 7.2, to iron-deficiency according to [42] using ethylenediamine-N,N′-bis-(2-hydroxyphenylacetic acid) (EDDHA), and to addition of 40 mM $CaCl_2$, 40 mM $MgSO_4$, as well as freeze-thaw cell lysates of 10^7 HeLa cells pro mL, respectively. These variations were carried out in the preculture as well as in the main culture (except of iron deficiency and cell lysates).

2.4 Production and harvesting Sop-proteins

The Sop proteins SopB, SopE1, SopD1, and AvrA have been purified from supernatants of respective *Salmonella* cultures. Cultures were transferred to the ice bath for 30 min. The supernatants were harvested by centrifugation (1 h, 20 000 rpm) and filtration through Millipore filter (0.45 µm). Precipitations of the proteins from the supernatants were carried out using 10% TCA on ice for 1 h. After 1 h centrifugation at 20 000 rpm respective precipitates were achieved and transferred to 0.4 mL 0.1 M NaOH and 2.0 mL ice-cold acetone (–20°C). After 20 min on –20°C, precipitates were harvested by centrifugation for 15 min at 20 000 rpm, washed again with ice-cold acetone, and harvested by centrifugation. The sediments were dried overnight, and then dissolved in 100 µL Laemmli buffer, heated for 5 min at 95°C and subsequently submitted to SDS-PAGE.

2.5 Purification and antibody production

The antibodies were raised in rabbits against the respective recombinant proteins which were produced using the pET-Directional TOPO® Expression Kit (Invitrogen BV), and purified using the ÄKTAexplorer NT100 (Amersham Bioscience, Freiburg, Germany), according to the manufac-

turer's instruction. Rabbits were injected subcutaneously with *ca.* 200 μg protein/doses and boostered 4 times (one booster per week).

2.6 SDS-PAGE and Western blots

SDS-PAGE was carried out according to [43] in a 10% polyacrylamide gel using the Mini-Protean™ apparatus (Bio-Rad, Hercules, CA, USA). Western blot analysis was performed as semidry blot using PVDF membranes and the Fast Blotter (Bio-Rad) for 15 min at 22 V/150 mA. For detection of Sop proteins, the polyclonal rabbit antibodies α-SopB, α-SopE1 (a gift from W.-D. Hardt), α-SopD1 and α-AvrA were applied. Since SopE1 as well as SopE2 proteins have been found to share positive reactions to the α-SopE1 antibody applied, their different molecular sizes and expression profiles allow identifying the SopE1 protein.

2.7 Quantitative estimation of the expression of effector proteins according to Western blot results

As outlined in Figs. 1a–1d, great quantitative differences in the Western blot signals concerning the effector proteins SopB, SopD1, SopE1, and AvrA were noted using the standard culture conditions noted above. In order to assess semiquantitatively the amount of proteins as detected by the Western blot technique we recorded them as follows: "–" no production; "(+)" very low level production (< 2 μg protein/mL); "+" low level production (2–5 μg protein/mL); "++" moderate production (5–10 μg protein/mL); "+++" strong production (>10 μg protein/mL); "0" the respective gene is not present, therefore no production.

3 Results

3.1 Production of the effector proteins (SopB, SopE1, SopD1, and AvrA) by various serovars of *S. enterica*

All studied 405 *S. enterica* isolates belonging to a great number of serovars (clinical strains, reference strains of the international SARB collection; see Section 2) have been identified to carry the *sopB* and *sopD1* genes whereas the genes *sopE1* and *avrA* were detected only among some of the serovars (Table 1). All of these clinical strains studied expressed the protein SopB and SopE1 under our "standard culture conditions" (see Section 2) but with quantitative differences (Table 1, compare Fig. 1d). The proteins SopD1 and particular AvrA (as far as the respective genes are present) were frequently found missing in culture supernatants (Table 1). A correlation of these quantitative differences with distinct RFLP types of the respective genes could not be seen (Table 1). In contrast to SopB, the production of the effector proteins SopD1, SopE1, and AvrA, respectively, appeared in correlation with the different clinical origins of the strains: serovars from systemic disease (such as *S.* Typhi, *S.* Paratyphi B, *S.* Choleraesuis, *etc*., also designated

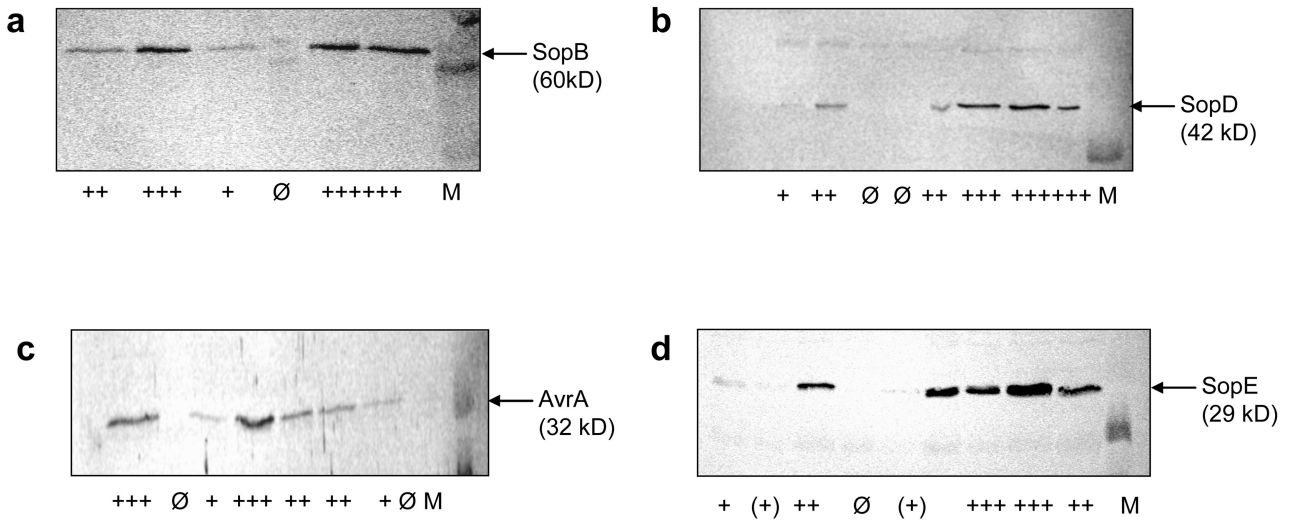

Figure 1. Illustration of quantitative differences in the expression profiles of *sopB*, *sopE1, sopD1*, and *avrA* determinants in various *S. enterica* variants according to Western blot signals. Western blot signals using α-SopB (a), α-SopD1 (b), α-AvrA (c), and α-SopE1 (d) antibodies, respectively, mean: –, no signal; (+), very weak signal: very low level production (correspond to <2 μg protein/mL); +, weak signal: low level production (correspond to 2–5 μg protein/mL); ++, moderate signal: moderate production (correspond to 5–10 μg protein/mL); +++, strong signal: strong production (correspond to >10 μg protein/mL); ∅, the respective gene is not present, therefore no respective proteins. M, molecular size standard.

Table 1. Presence, genetic polymorphism, and expression of effector protein genes *sopB*, *sopD1*, *sopE1*, and *avrA* among host-adapted, epidemic, and nonepidemic serovars of *S. enterica*

Origins and serovars	Number of strains tested	Classes of genetic polymorphisms [a]				Expression profiles (Western blot) [b]			
		sopB [c]	*sopD1* [d]	*sopE1* [e]	*avrA* [f]	SopB	SopD1	SopE1 [e]	AvrA
Systemic diseases									
Dublin	11	2	1	1	1	+++	+++	++	+
Gallinarum	13	2	2	3	1	++	+	–	–
Typhi	5	3	3	2	0	++	+	++	0
Paratyphi A	2	5	3	5	0	++	–	++	0
Paratyphi B (SPV) [g]	32	3	4	2	0	++	–	+++	0
Choleraesuis	19	8	5	6	0	+	–	–	0
Paratyphi C	3	10	5	0	0	+++	+	0	0
Typhisuis	3	14	5	0	0	–	–	0	0
Enteritis, nonepidemic									
Derby	7	7	1	0	1	++	+	0	–
Anatum	5	2	2	0	1	+	–	0	–
Blockley	4	3	2	0	1	++	++	0	–
Newport	7	2	5	0	1	+	–	0	–
Livingstone	3	12	4	0	–	+	–	0	0
Agona [h]	14	5	2	0	1	++	+	0	–
Paratyphi B (EPV) [g]	50	3	5	5	1/– [i]	++	–	+++	–/0 [i]
Heidelberg	8	8	2	4	1	+++	++	+++	–
Hadar	3	1	3	2	1	++	+++	+	–
Virchow [h]	9	13	4	0	1	++	+	0	– [j]
Bovismorbificans [h]	4	3	4	0	1	++	+++	0	–
Oranienburg [h]	15	3	4	0	1	+++	+++	0	–
Others [k]	66	+	+	0	+	++	+	0	–
Enteritis, epidemic									
Typhimurium	60 [l]	1	4	0/5 [i]	1	+++	+++	0/++ [i]	++
Enteritidis	42 [l]	2	2	6	1	+++	+++	+++	++

a) The numbers correspond to the presence as well as to the polymorphic classes of the respective genes and all strains belonging to the respective serovars appeared identical according to [33]; 0 means: no gene is present; the genes *sptP*, *sopE2*, *sipB*, and *sipC* are not considered here (compare [33]).
b) See Figure 1
c) Hybridization pattern of *Eco*RV-digested genomic DNA using *sopB* as a probe (signals with bands in kb): 14, 1.9 (type 1); 4.2, 1.9 (type 2); 4.2, 1.2, 0.7 (type 3); 14, 1.2, 0.7 (type 5); 4.2, 1.9, 0.7 (type 7); 1.9 (type 8); 2.1, 1.9 (type 10); 4.2, 2.5 (type 12); 4.2, 0.9 (type 13); 3.0, 2.1, 1.9 (type 14) according to [33].
d) Hybridization pattern of *Sal*I-digested genomic DNA using *SopD1* as a probe (signals with bands in kb): 19 (type 1); 16 (type 2); 14 (type 3); 21 (type 4); 5.0 (type 5) according to [33].
e) Hybridization pattern of *Pst*I-digested genomic DNA using *sopE1* as a probe (signals with bands in kb): 16 (type 1); 4.5 (type 2); 7.0 (type 3); 9.0 (type 4); 3.0 (type 5); 3.7 (type 6) according to [33]; *SopE1* can be differentiated from SopE2 due to its different molecular size.
f) Hybridization pattern of *Sal*I-digested genomic DNA using *avrA* as a probe (signals with bands in kb): 5.0 (type 1) according to [33].
g) SPV, see [34], EPV strains with different results, see Table 3.
h) Some isolates stem from epidemic outbreaks.
i) Heterogeneous within the serotype, see Table 3.
j) A single strain gave positive results.
k) *S.* Bareilly, *S.* Brandenburg, *S.* Duisburg, *S.* Emek, *S.* Haifa, *S.* Indiana, *S.* Infantis, *S.* Mbdaka, *S.* Miami, *S.* Montevideo, *S.* Muenchen, *S.* Panama, *S.* Reading, *S.* Rubislaw, *S.* Saintpaul, *S.* Schwarzengrund, *S.* Senftenberg, *S.* Stanley, *S.* Thomson, *S.* Tennesse, *S.* Wien
l) Various phage types and clonal types, see Table 3.

host-adapted serovars) have been characterized by their noticeable production of the SopB and SopE1 proteins, but with the absence of SopD1 and AvrA proteins (Table 1); the enteric serovars (with epidemic potential) appeared with the production of SopB, SopD1, but rarely AvrA (mainly observed among *S.* Enteritidis and *S.* Typhimurium). The nonepidemic *S. enterica* serovars (*e.g.*, *S.* Virchow, *S.* Agona, *S.* Derby, *etc.*) produced scarcely or not at all SopD1 and AvrA, respectively (Table 1). In particular, the very low level of expression of AvrA is remarkable and might be due to nonpermissive expression conditions in our standard culture procedure (see Section 2).

Table 2. Influence of different culture conditions on the expression of SopB, SopD1, and AvrA in "nonproducer" strains under the standard culture conditions

Serovar	Designation of effector protein	Production of effector proteins under different culture conditions							
		37°C standard	30°C	pH 6.0	pH 8.0	Fe deficiency	Ca^{++} add	Mg^{++} add	Cell lysates (HeLa)
S. Paratyphi B-EPV [a)]	SopB	+++	+++	+++	+++	+++	+++	+++	+++
	SopD1	–	–	++	–	–	–	–	–
	AvrA	–	–	+++	+	–	–	–	+
S. Paratyphi B-SPV [b)]	SopB	+++	++	++	++	++	++	++	++
	SopD1	–	–	–	–	–	–	–	–
S. Derby [c)]	SopB	++	++	++	++	++	++	++	++
	SopD1	–	–	–	–	–	–	–	–
	AvrA	–	–	–	–	–	–	–	–
S. Agona [d)]	SopB	++	++	+++	++	++	++	++	++
	SopD1	–	–	+	–	–	–	–	–
	AvrA	–	–	+++	–	–	–	–	+++
S. Enteritidis [e)]	SopB	++	+++	++	++	++	++	++	+++
	SopD1	–	+++	–	–	–	–	–	–
	AvrA	–	+	–	–	–	–	–	–
S. Bovismorbificans [f)]	SopB	++	++	++	++	++	++	++	+++
	SopD1	+++	++	++	++	++	++	++	++
	AvrA	–	–	++	+	–	–	–	–
S. Virchow [g)]	SopB	++	++	++	++	++	++	++	++
	SopD1	(+)	–	+++	–	–	–	–	–
	AvrA	(+)	(+)	+++	–	–	–	–	–
S. Hadar [h)]	SopB	++	++	++	++	++	++	++	++
	SopD1	+	?	+	–	?	++	?	+++
	AvrA	+	–	++	+	–	+	–	+++

a) Strain No: 00-01205, enteric pathovar according to [34] isolate from sporadic diarrhoea
b) Strain No: 99-09330, paratyphoid fever, *avrA* gene not present compare Table 1
c) Strain No: 02-03424, single isolate from pig
d) Strain No: 02-03414, epidemic strain from the *S.* Agona epidemic outbreak due to contaminated fennal-anise-careway tea-bags (Rabsch *et al.*, to be published)
e) Strain No: 02-03431, PT21 isolate from sporadic diarrhoea
f) Strain No: 97-12539, epidemic strain, probable associated
g) Strain No: 97-04315, sporadic diarrhoea with sprouts
h) Strain No: 00-03279, isolate from sporadic diarrhoea

3.2 Studies to induce expression of effector proteins AvrA and SopD1 from nonproducer strains of clinical origin

In contrast to SopB and SopE1 proteins which are produced well under our standard conditions from nearly all *S. enterica* strains in this study (Table 1, but see *S.* Typhisuis), the productions of the effector proteins SopD1 and AvrA happen with striking quantitative differences. These differences in the expression between SopB/SopE1 and SopD1/AvrA might be due to a different regulation of the respective genes rather than due to failures in the function of the type III secretion system (Table 2). Therefore, various culture conditions, such as variations in pH and temperature, presence of components from HeLa cells (well applied for *Salmonella* infections) various concentrations of Fe^{+++}, Mg^{++}, or Ca^{++} (see Table 2) were used in order to test a possible induction of the respective proteins. As summarized in Table 2, some of the SopD1 and AvrA nonproducer strains (*e.g.*, *S.* Paratyphi B and *S.* Agona, *S.* Bovismorbificans, *etc.*) began to produce AvrA and SopD under low pH culture condition whereas other strains (*e.g.*, *S.* Derby) remained negative (Table 2). Interesting to note, also cell extracts can induce the AvrA expression in *S.* Agona but not in *S.* Paratyphi B-EPV (Table 2).

Table 3. Expression of the effector proteins SopB, SopD1, SopE1, and AvrA among epidemic and nonepidemic strains (clones) of *S.* Typhimurium, *S.* Enteritidis, and *S.* Paratyphi B

Designation (numbers)[a]	Origin	Western blot signals			
		SopB	SopD1	SopE1	AvrA
S. Typhimurium[b]					
DT104 (6)	Epidemic strain since the nineties, enteritis	+++	+++	0	++
DT204 (24)	Epidemic strain of the seventies, severe enteritis	+++	+++	++	–
DT193 (5), DT120 (6)	Sporadic strain, enteritis	+++	+	0	+
DT40 (3)	Song bird-adapted strain	+++	–	0	+++
DT2 (6)	Pigeon-adapted strain[c]	++	++	0	+++
S. Enteritidis[d]					
PT4 (9)	Pandemic strain since the eighties in Europe, severe enteritis	+++	+++	+++	+++
PT21 (4), PT1 (3), PT6 (1)	Sporadic strains enteritis	+++	++	++	+
PT13 (4), PT8 (4)	Epidemic strains in North America	+++	+++	++	+
PT11 (4)	Nonhuman hedgehog-adapted	+	+	–	+++
S. Paratyphi B[e]					
EPV-1 (20)	Enteritis, sporadic cases, classical enteric variant	+++	+	0	–
EPV-2c (27)	Enteritis, single cases	+++	++	0	0
EPV-3 (15)	Emerging among poultry, avirulent for humans	++	0	0	–
SPV (50)	Human-adapted, typhoid fever, epidemics and single cases	+++	–	+++	0

a) Number of isolates tested
b) DT: phage type designation of *S.* Typhimurium; see [39]
c) It remains open why the pigeon adapt strains are very much similar to human epidemic strains in spite they do not occur in salmonellosis of men.
d) PT: phage type designation of *S.* Enteritidis; see [8]
e) Designation and classification according to [34]
0, no gene

3.3 Effector proteins expressed by host-adapted, epidemic strains as well as nonepidemic strains belonging to one serovar

The expression profiles of different clinical and epidemic strains were comparatively tested which belong each to a distinct serovar: *S.* Typhimurium, *S.* Enteritidis, and *S.* Paratyphi B. As summarized in Table 3, host-adapted variants used to produce SopB and SopE1 (either SopE1 or SopE2) but never or rarely SopD1 (except the pigeon-adapted *S.* Typhimurium DT2 strains), or AvrA (except the animal-adapted strains). Enteric variants of *S.* Typhimurium and *S.* Enteritidis, particularly those of epidemic origins, were characterized by a strong production of SopB, SopD1, and AvrA (except the epidemic *S.* Typhimurium DT204 strains which were very often associated with severe systemic-like gastroenteritis). In contrast, nonepidemic strains (*S.* Typhimurium DT193, *S.* Enteritidis PT1, *etc.*) among these three serovars were usually found with a low level expression of SopD1 and AvrA (see Table 3).

4 Discussion

The virulence factors of *S. enterica* responsible for inducing either focal, self-limiting enteritis or systemic infections (*e.g.*, typhoid fever) are just at the beginning to be understood [10]. However, the molecular background of the epidemic potency (epidemic virulence) of some of the *S. enterica* epidemic strains remain completely unknown as yet. There is growing evidence that two type III secretion systems, encoded by the pathogenicity island SPI1 and SPI2, respectively, mediate the translocation of an array of virulence-associated effector proteins directly into the target cell [12, 13]. These effector proteins can interfere with several metabolic functions of the host cell by modulating, inhibiting, or paralysing parts of the cellular network.

Although *S. enterica* is biologically a very diverse species with a large number of subspecies, serovars, and clonal types [4 – 8, 38, 39], not all variants are obviously able to cause disease or epidemic outbreaks. It turned out in the

past that this inability of many *S. enterica* strains cannot be explained by the absence of virulence genes [31, 33, 34, 37]. However, as outlined in Tables 1 and 3 (see Fig. 1) the expression profiles of the respective effector proteins under distinct conditions reveal differences between strains and serovars of different clinical and/or epidemic sources. For instance, strains of the host-adapted serovar *S.* Typhi (from systemic infections) are characterized by a strong expression of SopB and SopE1 proteins, but by missing SopD1 and AvrA proteins; in contrast, the epidemic enteritis strains of *S.* Typhimurium (*e.g.*, DT104) are characterized by their strong production of SopD1 and AvrA in addition to SopB. This appears to be similar to the epidemic strains of *S.* Enteritidis (Table 3). Interestingly, such differences between systemic and enteritis strains were also noted among the very heterogeneous serovar *S.* Paratyphi B (Table 3; see [34]). *S.* Paratyphi B strains with the typhoid fever potency (designated systemic pathovar, SPV; see [34]) have been characterized as strong SopB and SopE1 producers but with missing SopD1 and AvrA proteins. In contrast, the enteric variants of *S.* Paratyphi B (designated enteric pathovar, EPV) produce SopB, SopD1, and the acid-inducible protein AvrA (Table 2). This EPV is often found associated with diarrhoea, however, usually not occurring in epidemic outbreaks.

Since SopB is an inositol-phosphatase [22] interfering with a number of inositol-phosphate-based cellular regulations (inclusive water and electrolyte loss, inflammation, and actin rearrangements), it is regarded as an essential virulence protein. SopB acts in cooperation with other effector proteins, *e.g.*, with SopE1 (a CDC42 activator), which helps salmonellae to proceed in the invasion process [31], *e.g.*, with SopD1 which enables salmonellae to proceed in the enteritis "pathway". Although AvrA, a cysteine protease, interferes with the apoptosis pathway of the target cells [40], it must be also regarded as an effector protein involved in the enteritis pathway. The action of AvrA leads to a local apoptosis (scorched earth principle) and a stop of inflammation (IL8), thereby inhibiting the invasion pathway in salmonellosis [26, 31] and directing the enteritis pathway (see *S.* Typhimurium and *S.* Enteritidis in Tables 1 and 3).

Since the AvrA and SopD1 proteins have occurred as acid-inducible (Table 3) among some of *S. enterica* strains tested, in particular among these from the nonepidemic group (Table 1), the respective *Salmonella* strains might express their full virulence phenotype only under this specific environmental condition. This ought to be of relevance for food hygiene since several foods which have been found to be particularly risky for salmonellae [3], are acid foods (*e.g.*, mayonnaises and products of it). It was discussed earlier [41] that acidic conditions will induce an acid tolerance and an acid resistance of salmonellae in order to survive better in the acidic environment of the stomach. The results summarized in Tables 1 – 3 support the view that also some of the virulence-associated proteins will be better produced under this acidic condition. This might turn the respective strains into a more virulent state.

The data summarized here can be regarded only as the beginning of an understanding how the network of the virulence-associated effector proteins of salmonellae is regulated. More extensive studies including more strains and other effector proteins, are needed.

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