2. MATERIALS & METHODS

2.1. Bacterial Strains Used and Growth Conditions

2.1.1. Gram Negative Bacterial Strains Used

A spontaneous streptomycin resistant derivative of the invasive *Salmonella enterica* serovar Typhimurium (*Salmonella typhimurium*) strain ATCC 14028 was used throughout, but in four forms: the wild type (WT), two different isogenic insertion mutants (SLYAI and SLYAII), and a SlyA over expressing strain (SLYA++). Detailed accounts of the construction of these strains have been previously reported (strains SLYAI and SLYAII in Daniels et al, 1996 and strain SLYA++ in Ludwig et al, 1995), however, a brief description of the genetic changes will now be given. Strain SLYAI contains a plasmid insertion resulting in the C-terminal 8 codons (codons 139 to 146) being cut off from the *slyA* gene, and replaced by 45 fortuitous codons encoded by the vector DNA (Daniels et al, 1996). Since the insertional mutation in *S. typhimurium* strain SLYAI affected only the 3' terminal region of the *slyA* gene, it could not be excluded that this strain produced a partially functional SlyA. Therefore, a second mutant of *S. typhimurium* was used with an insertion in the 5' terminal portion of the *slyA* gene, named SLYAII. Strain SLYAII contained a mutated, certainly non-functional *slyA* gene that contained the N-terminal 42 codons directly fused to the C-terminal 8 codons but lacking the central 96 codons. Vector DNA follows the C-terminal 8 codons (Daniels et al, 1996). Strain SLYA++ comprises *S. typhimurium* WT transformed with pAL102, a recombinant derivative of the multicopy plasmid pBluescriptIISK$^+$ containing the complete *slyA* gene as insert (Ludwig et al, 1995).

All three derivatives of *S. typhimurium* 14028s described in this work, i.e. the *slyA* mutants, SLYAI and SLYAII, and *S. typhimurium* 14028s / pAL102 were morphologically identical to the wild type when grown on solid LB and MacConkey agar and exhibited similar growth rates to the wild type strain in liquid LB medium and MOPS-Glucose minimal medium at 37 °C (Daniels et al,

1996).

In the *Salmonella* loop invasion assays a *Yersinia enterocolitica* strain cured of it's virulence plasmid and containing an *inv* mutation (kindly provided by IB Autenrieth) was used as a negative control. Preparation of the inoculum of this strain was identical to that described below for the *Salmonella* strains. This *Y. enterocolitica* strain has a similar gentamicin minimum bactericidal concentration (MBC) as all the *S. typhimurium* strains used.

All strains were passaged through BALB/c mice by intraperitoneal (i.p.) inoculation, and two days later liver and spleen homogenates were cultured on Mueller-Hinton (M-H) agar. A single colony was picked and grown with shaking overnight at 37°C in Luria Betani (LB) broth containing 30µg of streptomycin per ml for the WT strain and 30µg of streptomycin per ml and 100µg of ampicillin per ml for the *slyA* mutants and the strain carrying pAL102. The over night cultures were diluted 1:20 with LB broth, and incubated for a further 4 hours until the logarithmic growth phase was reached. Subsequently the cells were pelleted by centrifugation (3850 g for 10 min), resuspended in LB broth containing 20 % (v/v) glycerol and frozen at -80°C. Colony forming unit (CFU) determinations were performed on M-H agar to determine viable counts of washed glycerol stocks. Before infection studies, bacteria were defrosted, washed twice with phosphate buffered saline (PBS), pH 7.4, and resuspended in the appropriate volume of PBS to yield the desired inoculum size.

2.1.2. Gram Positive Bacterial Strains Used

Three listeria strains were used: the laboratory *Listeria monocytogenes* strain EGD (in-house strain), an EGD strain containing an in-frame deletion in the *inlC* gene, termed Δ*inlC* (Engelbrecht et al, 1996), and *L. innocua* Sv 6a NCTC 11288 (National Collection of Type Cultures, London, England). All *L. monocytogenes* strains were passaged through BALB/c mice before infection studies were performed, as described for the Gram negative strains above. Isolated colonies were grown in Brain Heart Infusion (BHI) broth overnight, spun and

resuspended in 20 % (v / v) glycerol in BHI broth. These stocks were frozen and kept at -80 °C until use. Just before mice, or cells, were infected, an aliquot was defrosted, washed twice in PBS and resuspended in PBS to yield the desired inoculum size.

As a control organism in certain loop invasion assays a strain of *Bacillus subtilis* (in-house strain) was used, but this was not passaged through mice due to its total avirulence, and overnight cultures were freshly prepared using BHI broth for each experiment, ie it was not frozen in glycerol.

2.2. Protein Studies of *Salmonella typhimurium*

2.2.1. Isolation of Cytoplasmic Proteins

The cells of 10 ml culture grown to the mid logarithmic or late stationary phase were collected by centrifugation, washed twice in PBS to remove extracellular proteins, resuspended in 500µl double strength Laemmli sample buffer and boiled for 4 min to denature the proteins. The resulting solution was spun to remove particulate matter and the supernatant was stored at -20˚C until use.

2.2.2. Isoelectric Focusing of Proteins (First Dimension)

The two dimensional electrophoresis method used in this work is the recommended method from BioRad (Bio-Rad Laboratories, Hercules, CA, USA) using the Protean II xi 2-D gel system. The exact method can be found in the BioRad Protean II xi 2-D users manual, but will be briefly summarised here. The 18.0 cm long, 1.5 mm internal diameter glass tubes were cleaned in 70 % (v / v) ethanol containing 10 % (w / v) KOH overnight, washed thoroughly in distilled water and allowed to dry in a 37°C incubator. The gels were accurately filled to a height of 13.0 cm with degassed gel mixture (see later) and left at room temperature for 2 h to polymerise. The denatured protein samples were diluted 1:10 with iso-urea solution E, spun to remove any particulate matter and 30 µg of the supernatant loaded onto the surface of the tube gel. This was carefully overlayed with 20 mM NaOH (upper running buffer) and the tube placed into the

gel chamber. This was repeated for every sample to be run, and triplicates of each sample were run simultaneously. After adding the upper (20 mM NaOH) and lower (10 mM H_3PO_4) running buffers, voltage was applied with the following programme: 2 h at 200 V, 2 h at 500 V, 16 h (overnight) at 800 V.

The glass tubes were removed from the gel chamber, thoroughly washed with distilled water and the tube gels were extracted into 10 ml plastic sample tubes. Any excess fluid was drained and the sample tubes were placed immediately in methanol containing dry ice (~ -70°C). After a few minutes the tubes were placed in a freezer at -20 °C until use.

A few minutes before loading a tube gel onto an SDS-PAGE slab gel, the tube gels were quickly defrosted and treated with 2.0 ml SDS-transfer solution for 3 min at room temperature. The second dimension was carried out as stated below, but non discontinuous (continuous) gels were used, ie no stacking gel.

2.2.3. SDS-PAGE Analysis of Proteins (Second Dimension)

SDS-PAGE of the extracted proteins was performed essentially as described by Laemmli (1970), but using the apparatus and methods from BioRad (Protean II xi Cell). Briefly, a 1.0 mm or 0.75 mm thick discontinuous gel was cast using a 12.0 % separating gel overlayed with a 4.0 % stacking gel. The gel was loaded with appropriate protein suspensions and a weight marker (Broad Range SDS-PAGE weight standard, Bio-Rad Laboratories, Hercules, CA, USA) for comparison and run for 1-2 h at 200 V or, for the second dimension (see above), at a fixed current of 15-20 mA. The gels were then either examined with coomassie blue staining, silver staining or western blot analysis (see below).

2.2.4. Coomassie Blue Staining

Gels were placed in fixative (50 % Methanol, 10 % Acetic acid) or stained immediately with Coomassie Blue (0.1 % w / v Coomassie Blue R-250 in fixative) for 30 min. The gels were then destained with fixative for 1-3 h and then placed in 10 % acetic acid. The stained gels remained in 10 % acetic acid or were vacuum dried.

2.2.5. Silver Staining

Freshly run slab gels were placed in fixative (50 % Methanol, 12 % Acetic acid) for 60 min or more, washed with 30 % ethanol for 20 min and placed in oxidiser (0.865 mM $Na_2S_2O_3.5H_2O$, 8.95 mM Paraformaldehyde) for 1 min. After three 1 min washings with distilled H_2O the gels were placed in freshly made silver nitrate solution (12 mM $AgNO_3$, 8.95 mM Paraformaldehyde) for 25-30 min, then washed twice for 30 s in distilled H_2O. The freshly made developer (0.57 M Na_2CO_3, 17.3 µM $Na_2S_2O_3.5H_2O$, 6.72 mM Paraformaldehyde) was added until most protein spots could be seen (5-15 min). The gels were then quickly placed in distilled H_2O (two washes for 2 min each) and the staining reaction stopped with fixative solution. The gels could then be left in fixative, or dried with a gel vacuum dryer.

2.2.6. Western Blot Analysis

Whole cell proteins of *S. typhimurium* strains were separated by SDS-PAGE and analysed by immunoblotting using a rabbit derived polyclonal anti-SlyA antiserum (Ludwig et al, 1995). The separated proteins were transferred to a nitrocellulose filter according to the method of Towbin et al (1979). Subsequently, the proteins were probed with anti-SlyA antibodies (1:1000), and bound antibodies were detected by addition of anti-rabbit horseradish peroxidase conjugated immunoglobulins (1:1000; Jackson ImmunoResearch Laboratories Inc,West Grove, PA, USA), followed by colourimetric development with chloronaphthol/H_2O_2. The filter was subsequently washed once with sterile distilled water and left over night at 4˚C. It was found that the colourimetric reaction could be slightly enhanced by leaving the washed filter in water over night at 4˚C [1].

2.3. Animal Experimental Methods

The experiments described in this work were performed within the framework of permission granted by the Regierung von Unterfranken (ref no: 621-2531.01-

[1] Unpublished observation by Zeljka Sokolovic.

47 / 96). The experiments were designed to obtain the most data from the least number of animals possible, and that the animals would suffer as little pain / discomfort as possible for the minimum feasible duration.

6-8 week old female BALB / c mice or 8-10 week old C57 / BL6 mice (Charles River Wiga, Sulzfeld, Germany) were used, and kept in positive pressure cabinets under specific pathogen free conditions. Mice were fed with food and water *ad libitum* unless otherwise stated. Normally, 5 mice were used for each bacterial strain at each time point for each experiment, and each experiment was performed at least twice. Throughout each experiment mice were checked at least once per day, and any dead mice (dead through the experimental infection) were removed, although this occurrence was very rare.

2.3.1. Ligated Ileal Loop Test

Mice were starved for 18 h prior to the experiment. Anaesthesia was accomplished with an i.p. injection of 1.8-2.0 mg of Nembutal (Rhone Merieux, Laupheim, Germany) per mouse, and when the deep stage of anaesthesia was reached a small, midline incision (1-1.5 cm) was made down the abdomen so that the small intestine was revealed. To form the loop two ligations were made in the ileum, leaving 3-5 cm gap between each ligation; each loop contained at least one Peyer's patch (see Fig. 2.1). The blood supply to the small intestine was always carefully preserved. 100 µl of bacterial suspension was injected into the lumen of the ligated intestine via a 0.4 mm needle, and the small intestine was returned to the bowel. The incision was closed with surgical thread, and the mice were kept alive for between 15-180 min. After this the mice were killed by cervical dislocation or asphyxiation by CO_2, and the tissue was processed as stated below.

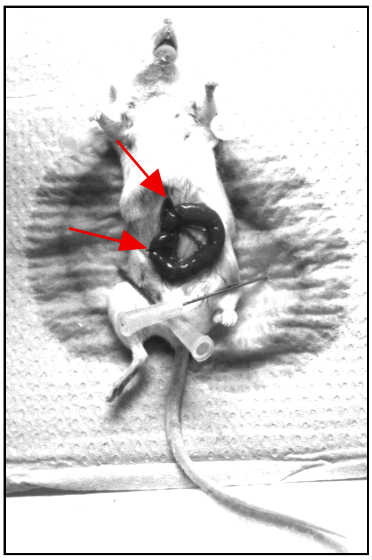

Fig 2.1. Ileal loop procedure. The two ligations can be seen (arrows). The bacter suspension is injected through the gut wall into the lumer and the ligated gut is returned to the abdominal cavity.

2.3.2. Ileal Loop Invasion Assay

Ligated ileal loop tests were performed as described above and after 90 min, the mice were killed, the abdomen reopened, the ligated intestine removed, and cut open longitudinally opposite the Peyer's patch. The tissue was then pinned flat on a block of wax, and gently washed with ice cold PBS to remove mucous and excess bacteria. The Peyer's patches were excised with the aid of a tissue punch, placed in LB broth containing gentamicin (100 µg per ml), and incubated for 60 min at 37°C with shaking to kill extracellular bacteria. Then, the tissues were thoroughly washed in ice cold PBS to eliminate residual gentamicin, and homogenised for CFU determination (see later).

2.3.3. Orogastric Inoculation

Before infection mice were starved for 18 h to clear the contents of the bowel. Mice were then fed 100µl of bacterial suspension via a flexible orogastric feeding tube. A metal grid was placed in the cages of the orogastrically infected mice that separates the bottom of the cage from the mice to prevent kapography.

2.3.4. Intravenous Inoculation

Mice were placed under an infrared lamp to warm the skin and hence to better reveal the tail veins. They were then infected with 100 µl of bacterial suspension using a 1 ml syringe with a 0.4 mm needle via one of the lateral tail veins.

2.3.5. Intraperitoneal Inoculation

Mice were infected with 100 µl bacterial suspension using a 1 ml syringe with a 0.4 mm needle by injecting into the middle of the lower part of the abdomen, from the under side, into the abdominal cavity.

2.3.6. Obtaining Blood Via Heart Puncture

Mice were either killed by cervical dislocation or asphyxiation with CO_2, or were under deep anaesthesia, when the rib cage was carefully cut open, a 0.4 mm needle inserted into the heart and blood was then extracted. An average of 1 ml was obtained with this procedure. If the mouse was under anaesthesia, it was

then quickly killed.

2.3.7. Removing Organs for Further Analysis

Mice were killed by either cervical dislocation or asphyxiation with CO_2, pinned flat on a dissecting board and sprayed with 70 % (v/v) ethanol. Using sterile surgical instruments the abdomens were then opened and the organs, eg liver, spleen, small intestine, carefully removed. The liver was always removed last as one has to cut major arteries and veins which leads to contamination by blood of all remaining organs in the abdomen. If Peyer's patches and/or pieces of the small intestine were needed, the small intestine was first washed out with 10 ml PBS, and then the required tissue was carefully removed using surgical scissors or a tissue punch.

2.3.8. Estimating Bacterial Loads in Organs

Organs were placed in 5 ml sterile distilled water and homogenised using 15 ml glass homogenisers for livers and spleens, and 5 ml glass homogenisers for Peyer's patches and small intestine pieces. Serial dilutions of the homogenates were then plated onto appropriate agar, and incubated at 37°C. CFUs per organ or per Peyer's patch/small intestine piece were then calculated for each mouse, and statistically tested for significant differences (see later). non-infected mice were also routinely killed as a negative control- in all cases no bacteria grew from organ homogenates.

2.4. Tissue Culture

All tissue culture work was performed in a Class II biological safety cabinet with the normal considerations regarding propagation, sterility, etc (source: ATCC web site: http://www.atcc.org./faqs.html). A 37°C incubator supplemented with 5 % CO_2 was used throughout.

2.4.1. Conversion of Human Enterocytes into M Cells by Peyer's Patch Lymphocytes

This method has been described in detail by Kernéis et al (1997) and is also summarised in Fig. 2.2. Briefly, a specific sub clone of the Caco-2 immortal cell

line was used (Caco-2 clone 1, Peterson and Mooseker, 1992) as this clone produces a particularly pronounced brush border (long microvilli) which more closely resembles the cell morphology of real *in situ* enterocytes in the mouse and human. 3 x 10^5 of these cells, in 100 µl, were seeded on inverted Transwell filters (6.5 mm diameter, 3 µm pore size; Costar GmbH, Bodenheim, Germany) and incubated in a sterile, moist container for 6 h at 37°C. The filters were then placed in the correct orientation in a 24 well tissue culture plate with the recommended amount of medium (600 µl in the lower chamber, 100 µl in the upper chamber). The cells were grown on these filters for 14 days with regular (3 times weekly) changes of medium containing 100 µg / ml streptomycin and 100 U / ml penicillin. On day 14 lymphocytes were isolated from surgically removed Peyer's patches of 8-10 week old female BALB / c mice. A single cell suspension was made by squeezing the Peyer's patches between two sterile glass slides in the presence of PBS (no Ca^{++} and Mg^{++}). The resulting cell suspension was then washed in medium containing 100 µg / ml streptomycin and 100 U / ml penicillin and resuspended in medium to yield 10^7 cells / ml. 100 µl of the cell suspension was added to the upper chamber of the Transwell filters and incubated for a further 4 days. On the fourth day transepithelial resistance was measured (Millicel-ERS; Millipore, Bedford, MA, USA)) for every Transwell. The average and standard

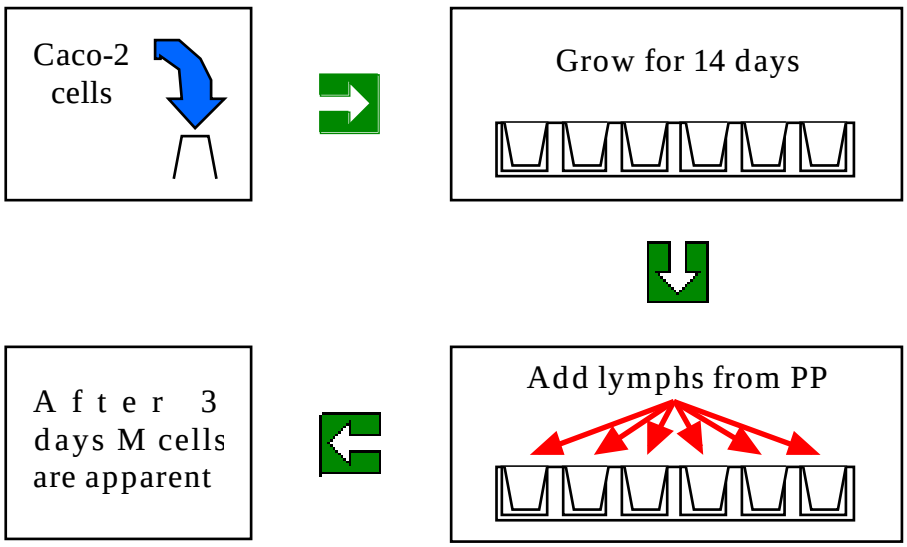

Fig 2.2. Diagramatic summary of co-culture method used to form M cells in vitro. After M cell formation has occurred, the cells are ready for infection.

deviation was calculated for each batch of Transwells and any Transwells that exhibited a deviation of more than 1 standard deviation from the average were discarded. This produced a more homogeneous batch of cells and also discarded any that had not formed a tight monolayer. Immediately after this the medium was changed to one without any antimicrobial activity. One day later the cells were ready for infection.

2.4.2. Cell Invasion Assays

The commonly used gentamicin survival assay was used: after infection of the cells for 1 h with ~10^7 CFU bacteria (MOI≈100), cells were washed with PBS to remove non-adherent bacteria and incubated with medium supplemented with gentamicin to a final concentration of 100 µg / ml for a further 1 h. After this the cells were washed three times with ice cold PBS to remove the gentamicin, and lysed using 1 ml sterile, ice cold, distilled water. This suspension was diluted in PBS and plated onto BHI agar for CFU determination. Statistical analyses were performed as described later in this work. As negative controls for all invasion assays, non-infected monolayers, and infected Transwells without cells, were processed in the above manner and in all cases grew no bacteria.

2.5. Electron Microscopy

The methods described for electron microscopy are those recommended by the Department of Electron Microscopy in the Biozentrum of Würzburg University, Germany.

2.5.1. Transmission Electron Microscopy

Samples were fixed with ice cold half strength Karnovsky reagent (Karnovsky, 1965), supplemented with 1 % Dimethyl sulphoxide (DMSO) to allow better penetration. The samples were then post fixed with 2 % osmium tetroxide (Electron Microscopy Sciences, Washington, DC) for 5 h at 4°C, and then placed in 0.5 % uranyl acetate (Merck) overnight at 4°C. The following day samples were dehydrated through a series of alcohol dilutions (50-100 %), and then placed in

propylene oxide for 30 min. Samples were infiltrated with epon embedding resin (Epon 812, Serva, Heidelberg, Germany), and incubated at 60°C for 48 h. Semi thin sections (1-2 µm) were made to locate the desired areas of the sample, and then ultra thin sections (60-80 nm) were cut and stained with uranyl acetate in alcohol and lead citrate. Finally, the samples were examined using a Zeiss EM10 transmission electron microscope at 80 kV (Zeiss, Oberkochen, Germany).

2.5.2. Scanning Electron Microscopy

Samples were washed in ice cold PBS and placed in ice cold 2 % (v/v) glutaraldehyde in Sorensen's buffer (pH 7.4) overnight at 4°C. The following day samples were dehydrated through a series of acetone dilutions (35-100 %), and finally dehydrated with a critical point dryer (CPD 030; BAL-TEC, Walluf, Germany). Samples were gold sputtered (SCD 005; BAL-TEC) to a thickness of 30 nm, and examined using a Zeiss Digital Scanning Microscope (DSM 962; Zeiss, Oberkochen, Germany).

2.6. Confocal Laser Scanning Microscopy

The confocal laser scanning microscope (CLSM) allows higher resolution than conventional fluorescent microscopes, and the ability to focus on a particular plane allowing a virtual cross sectional analyses within the cells. For these two reasons CLSM was used throughout this work. Standard methods were employed which are described in detail in Ojcius et al (1996). The samples were then examined using a CLSM from Leica Lasertechnik GmbH (Heidelberg, Germany) with Leica TCS NT vers 1.00 software. Images were then further processed using NIH Image v1.61PPC (National Institutes of Health, USA; web site: http://rsb.info.gov/nih/image/) and GraphicConverter v3.6 (Lemke Software, Peine, Germany; web site: http://www.lemkesoft.de/) on an Apple™ PowerMac.

2.6.1. Fluorescent staining techniques

The antibodies and fluorescent agents used are listed in Table 2.1. Standard staining methods were employed, using the following protocol:

Samples were fixed using either 4.0 % paraformaldehyde (containing 120 mM sucrose in PBS, pH≈7.0) overnight at 4 ˚C, or in 100 % methanol at -20 °C for 30 min. The samples are then washed three times in PBS and placed in 50 mM NH_4Cl-PBS (quenching reagent) for 15 min at room temperature (RT) and then further washed twice with PBS. If intra- and extracellular bacteria were wanted to be visualised, then permeabilisation of the cells was accomplished by incubating with 0.2 % Triton X100 in PBS for 4 min at RT and then washing three times with PBS. To reduce non-specific antibody interactions the cells were incubated for 20-30 min in 0.1 % bovine serum albumin in PBS (BSA-PBS), and all antibodies and dyes were also diluted in this blocking reagent. The first antibodies, and/or conjugated probes, were then added for 60 min at RT- the volume can normally be reduced to a minimum so that the sample is just covered. The samples were then washed with BSA-PBS three times and the second antibodies were added for

Probes For	**Probe Name**	**Source Organism**	**Working Dilution**	**Source**
Listeria monocytogenes	Poly-clonal anti listeria Ab	Rabbit	1:50	Produced in-house, by Guido Dietrich
Actin cytoskeleton of eukaryotic cells	Phalloidin, conjugated with TRITC	N/A	1:20	Sigma, St. Louis, MO, USA
Actin cytoskeleton of eukaryotic cells	Phalloidin, conjugated with FITC	N/A	1:20	Sigma, St. Louis, MO, USA
Sucrase iso-maltase-a specific marker of differentiated columnar epithelial cells, and not M cells	Anti-sucrase iso-maltase Ab, conjugated with FITC	Rabbit	1:500	Kernéis et al, 1997
Fucose, found in the apical membranes and glycocalyx of murine M cells	UEA-1, labelled with FITC	Ulex europaeus 1	1:40	Sigma, St. Louis, MO, USA
As above	UEA-1, labelled with TRITC	Ulex europaeus 1	1:40	Sigma, St. Louis, MO, USA
Rabbit IgG, whole molecule	Anti-Rabbit Ab, conjugated with TRITC	Goat	1:40	Jackson Immuno-Research Laboratories Inc, West Grove, PA, USA
Rabbit IgG, whole molecule	Anti-Rabbit Ab, conjugated with FITC	Goat	1:40	
Rabbit IgG, whole molecule	Anti-Rabbit Ab, conjugated with Cy-5	Goat	1:40	

Table 2.1. List of antibodies and flurescent dyes used in immuno-staining. Ab=antibody FITC=fluorescein isothiocyanate, TRITC=tetramethylrhodamine isothiocyanate, UEA-1=lectin from *Ulex europaeus*-1, N/A=not applicable.

a further 60 min. After the last wash step (with BSA-PBS), all excess fluid was drained from the sample using a cotton wool bud and a drop of 50 % glycerol in PBS was placed over the sample. A cover slip was positioned over the sample and after 1 h in the dark at 4 °C nail varnish was used to seal the edges. Samples could be kept in the dark at 4 °C for a few weeks.

2.7. Statistical and Graphical Methods

Numerical results are given as arithmetic means ± standard deviations of the means. CFU determinations were converted to $\log_{10}$ values and then the arithmetic means and standard deviations calculated. All graphs use a linear scale on the y axis and the plotted values are all log values, except where indicated. The Student's t Test (two tailed, unpaired) was used to test statistical significance, and P values of less than 0.05 (5 % confidence) were considered statistically significant. The mathematical formula is expressed as follows, and the calculated T value is then compared with standard table T values- if the table T value is greater than the calculated value for the given confidence limit and degrees of freedom (k) the two samples very probably ($P \geq 0.95$) come from different populations. That is to say, there is a statistically significant difference between the two samples.

All statistical analyses and graphs were generated using ClarisWorks v5.0 (Claris Corporation, USA) on an Apple™ PowerMac computer.

$$T = \frac{\bar{X} - \bar{Y}}{\sqrt{(n_1 - 1)S_X^2 + (n_2 - 1)S_Y^2}} \times \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2}}$$

and

$$k = n_1 + n_2 - 2$$

where:

$\bar{X}$ and $\bar{Y}$ = the arithmetic means of samples X and Y, respectively.

S_x and S_y = the standard deviations of samples X and Y, respectively.

n_1 and n_2 = the sample size of samples X and Y, respectively.

Fig 2.3. The mathematical formula used to calculate the T value from two groups of data. The calculated T value is then compared with values found in statistical tables.

2.8. Chemicals and Media

All chemicals were obtained from Sigma (Sigma, St. Louis, MO, USA) or Merck (Merck KGaA, Darmstadt, Germnay), and used interchangeably unless otherwise stated in the text.

2.8.1. General Chemicals and Solutions

Phosphate Buffered Saline (10x stock solution)

12.36 g Na_2HPO_4, anhydrous
1.80 g $NaH_2PO_4.H_2O$
85.00 g NaCl
make up to 1000 ml and autoclave
Dilute 1:10 with steril distilled H_2O (yields a 0.01 M Phosphate buffer, pH 7.6

2.8.2. Electrophoresis Chemicals and Solutions

Laemmli Sample Buffer
50 mM Tris-HCl pH 6.8
10 % (v / v) glycerol
5 % (v / v) β-mercaptoethanol
2 % (w / v) SDS
0.05 % (w / v) bromophenol blue

Iso-Urea Solution E
0.1 g DTT
0.4 g CHAPS
5.4 g Urea
500 µl ampholyte, pH 3-10
6 ml Distilled H_2O

Detergent Solution
0.3 g CHAPS
100 µl Nonidet P-40
900 µl Distilled H_2O

First Dimension IEF Gel

Makes 20 ml- enough for 16 1.0 mm tube gels.

11 g Urea

3 ml 30 % Acrylamide / bis (Rotiphorese Gel30, Carl Roth GmbH, Karlsruhe, Germany)
0.5 ml ampholyte, pH 3-10
0.25 ml ampholyte, pH 5-8
0.25 ml ampholyte, pH 4-6.5
1 ml Detergent Solution (see above)
Deionised H_2O to 20 ml
Degas for 5-10 min
20 µl TEMED
40 µl 10 % (w / v) Ammonium persulphate

SDS-Transfer Solution
40 ml 0.5 M Tris-HCl, pH 8.8
80 ml 10 % SDS
8 ml 0.05 % bromophenol blue
150 ml deionised H_2O

Second Dimension SDS-PAGE Separating Gel (12 %)

Makes 100 ml- enough for two slab gels.

33.5 ml Distilled H_2O
25.0 ml 1.5 M Tris-HCl, pH 8.8
1.0 ml 10 % (w / v) SDS
40 ml Acrylamide / bis 30 % (Rotiphorese Gel30, Carl Roth GmbH, Karlsruhe, Germany)
500 µl 10 % (w / v) Ammonium persulphate
50 µl TEMED

2.8.3. Growth Media

All media was purchased from Oxoid (UniPath Ltd, Hampshire, UK), except where indicated, and made according to the manufactures instructions.

Mueller Hinton Agar Oxoid code CM337

Salmonella-Shigella Agar Oxoid code CM533

Listeria Selective Agar Listeria agar base (Oxford), Oxoid code CM856

Listeria selective supplement (Oxford), Oxoid code SR140E

MacConkey Agar With lactose, without NaCl, Oxoid code CM7b

Luria Bertani (LB) Broth/Agar

10 g Peptone (Gibco, Life Technologies, Paisley, Scotland)

5 g Yeast extract (Gibco)

10 g NaCl

make up to 1000 ml with distilled H_2O

adjust to pH 7.0 with NaOH (1.0 M)

If agar is required, add 15 g Agar (bacteriological)

Brain Heart Infusion (BHI) Broth/Agar

BHI (Merck)

If agar is required, add 15 g Agar (bacteriological)