

Supplementary Information for

Foodborne pathogen dynamics in meat and meat analogue analysed by traditional microbiology and metagenomic sequencing

Supplementary Table S1. Equations to determine cfu/mL from optical density at 600nm.

| Bacteria | Equation to determine colony-forming units |
|---------------------------------|---|
| <i>Listeria monocytogenes</i> | $Y = (12.98 \times 10^8) X - 1.71 \times 10^6$ |
| <i>Salmonella</i> Typhi | $Y = (6.895 \times 10^8) X - 0.319 \times 10^6$ |
| <i>Escherichia coli</i> 0157:H7 | $Y = (5.841 \times 10^8) X - 5.04 \times 10^6$ |

Y = Colony forming units; X = Optical density at 600nm.

Supplementary Table S2. Optical density at 600nm and corresponding colony forming units used for spiking in pig, pea, and soy juice.

| Microorganisms | OD | CFU/mL |
|--------------------------------|-------|--------------------|
| <i>Listeria monocytogenes</i> | 0,209 | $2,6 \times 10^8$ |
| <i>Salmonella</i> Typhi | 0,588 | $4,05 \times 10^8$ |
| <i>Escherichia coli</i> HEHA16 | 0,724 | $4,1 \times 10^8$ |
| <i>Cronobacter sakazakii</i> | 0,461 | $2,6 \times 10^8$ |

OD = Optical density at 600 nm.

Supplementary Table S3. Optical density at 600nm and corresponding colony forming units used for spiking in beef and chicken juice.

| Microorganisms | OD | CFU/mL |
|--------------------------------|-------|-------------------|
| <i>Listeria monocytogenes</i> | 0,209 | $2,6 \times 10^8$ |
| <i>Salmonella</i> Typhi | 0,604 | $4,1 \times 10^8$ |
| <i>Escherichia coli</i> HEHA16 | 0,538 | $3,1 \times 10^8$ |
| <i>Cronobacter sakazakii</i> | 0,318 | $1,8 \times 10^8$ |

OD = Optical density at 600 nm.

Supplementary Table S4. Bacterial concentrations expressed as cfu/100 µL in the 5 mL meat/meat analogue juices.

| Food matrix | <i>Escherichia coli</i> | | | |
|-------------|-------------------------|-------------------------------|-------------------------|------------------------------|
| | HEHA16 | <i>Listeria monocytogenes</i> | <i>Salmonella</i> Typhi | <i>Cronobacter sakazakii</i> |
| PIG | 4,1 x 10 ⁶ | 2,6 x 10 ⁶ | 4,05 x 10 ⁶ | 2,6 x 10 ⁶ |
| PEA | 4,1 x 10 ⁶ | 2,6 x 10 ⁶ | 4,05 x 10 ⁶ | 2,6 x 10 ⁶ |
| SOY | 4,1 x 10 ⁶ | 2,6 x 10 ⁶ | 4,05 x 10 ⁶ | 2,6 x 10 ⁶ |
| CHICKEN | 3,1 x 10 ⁶ | 2,6 x 10 ⁶ | 4,1 x 10 ⁶ | 2,6 x 10 ⁶ |
| BEEF | 3,1 x 10 ⁶ | 2,6 x 10 ⁶ | 4,1 x 10 ⁶ | 2,6 x 10 ⁶ |

Supplementary Table S5. Relative abundances of the detected pathogenic bacterial taxa using metagenomic sequencing in all included meat and meat analogues where a single bacterium was inoculated into the matrix and incubated over time. 1, 2 and 3 represent the 3 days of incubation, day 1, day 2 and day 3.

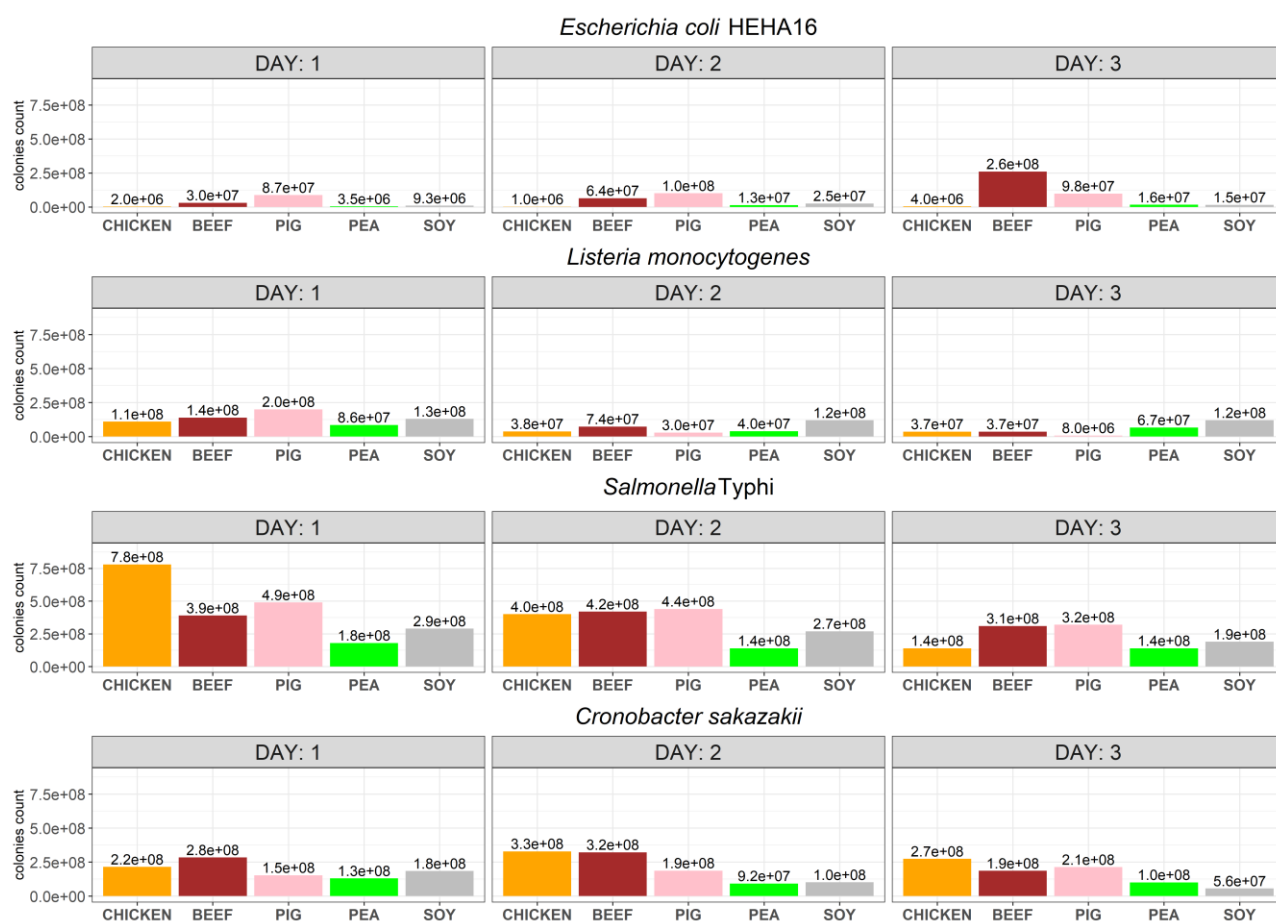
| <i>C. sakazakii</i> | | <i>E. coli</i> | | <i>L. monocytogenes</i> | | <i>S. Typhi</i> | |
|---------------------|----------|----------------|----------|-------------------------|------------------------|-----------------|----------|
| Beef Crono 1 | 89,14434 | Beef Coli 1 | 90,67842 | Beef List 1 | 92,0036 | Beef Salmo 1 | 82,51322 |
| Beef Crono 2 | 88,86935 | Beef Coli 2 | 87,20641 | Beef List 2 | 93,38248 | Beef Salmo 2 | 79,89853 |
| Beef Crono 3 | 89,15337 | Beef Coli 3 | 86,54829 | Beef List 3 | 93,38008 | Beef Salmo 3 | 79,06574 |
| Chicken Crono 1 | 86,75402 | Chicken Coli 1 | 13,06994 | Chicken List 1 | 75,53367 | Chicken Salmo 1 | 77,8446 |
| Chicken Crono 2 | 88,61094 | Chicken Coli 2 | 6,24416 | Chicken List 2 | 73,94991 | Chicken Salmo 2 | 78,99075 |
| Chicken Crono 3 | 87,65216 | Chicken Coli 3 | 26,41271 | Chicken List 3 | 83,52631 | Chicken Salmo 3 | 78,215 |
| Peas Crono 1 | 89,80104 | Peas Coli 1 | 84,51989 | Pig List 1 | 78,01425 | Peas Salmo 1 | 90,04712 |
| Peas Crono 2 | 89,77533 | Peas Coli 2 | 86,75854 | Pig List 2 | 92,48886 | Peas Salmo 2 | 90,58372 |
| Peas Crono 3 | 89,66048 | Peas Coli 3 | 86,49998 | Pig List 3 | 93,01339 | Peas Salmo 3 | 90,49974 |
| Pig Crono 1 | 87,15304 | Pig Coli 1 | 82,71754 | Soy List 1 | 8,67433 | Pig Salmo 1 | 90,03666 |
| Pig Crono 2 | 88,81244 | Pig Coli 2 | 81,1363 | Soy List 2 | DNA was too fragmented | Pig Salmo 2 | 89,46737 |
| Pig Crono 3 | 89,06832 | Pig Coli 3 | 82,93077 | Soy List 3 | DNA was too fragmented | Pig Salmo 3 | 89,4086 |
| Soy Crono 1 | 87,13998 | Soy Coli 1 | 88,05545 | Peas List 1 | DNA was too fragmented | Soy Salmo 1 | 87,93873 |
| Soy Crono 2 | 88,46208 | Soy Coli 2 | 91,74899 | Peas List 2 | DNA was too fragmented | Soy Salmo 2 | 89,06861 |
| Soy Crono 3 | 88,4132 | Soy Coli 3 | 93,32131 | Peas List 3 | DNA was too fragmented | Soy Salmo 3 | 88,81941 |

Supplementary Table S6. Relative abundances of the detected pathogenic bacterial taxa using metagenomic sequencing in all included meat and meat analogues where a cocktail of the four tested bacteria was inoculated into the matrix and incubated over time. 1, 2 and 3 represent the 3 days of incubation, day 1, day 2 and day 3.

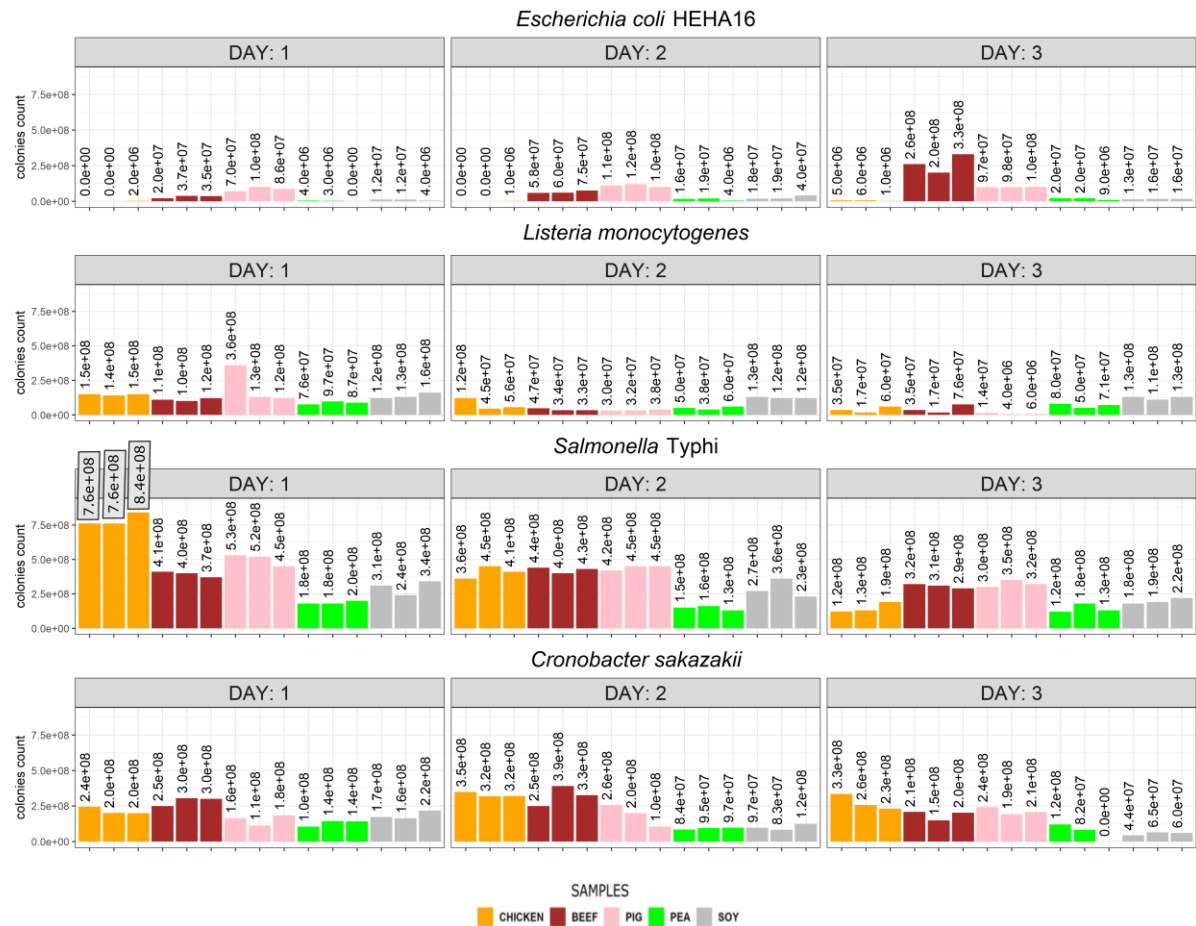
| | <i>C. sakazakii</i> | <i>E. coli</i> | <i>L. monocytogenes</i> | <i>S. Typhi</i> |
|---------------|---------------------|----------------|-------------------------|-----------------|
| Beef Mix 1 | 37,33244 | 4,92845 | 2,05032 | 41,67876 |
| Beef Mix 2 | 28,36945 | 1,88599 | 0,34616 | 53,35242 |
| Beef Mix 3 | 25,02477 | 1,4614 | 0,3375 | 55,63213 |
| Chicken mix 1 | 16,07143 | 9,19263 | 0,40249 | 40,41137 |
| Chicken Mix 2 | 20,70064 | 0,1614 | 0,1833 | 55,94276 |
| Chicken mix 3 | 21,02403 | 0,14638 | 0,31021 | 56,65993 |
| Peas Mix 1 | 43,17743 | 7,05854 | 2,5974 | 36,20287 |
| Peas Mix 2 | 40,2535 | 6,46692 | 1,40915 | 41,49422 |
| Peas Mix 3 | 42,50316 | 7,17402 | 2,16702 | 38,37138 |
| Pig Mix 1 | 30,19369 | 5,49055 | 1,11744 | 52,31025 |
| Pig Mix 2 | 23,54396 | 5,57115 | 0,51391 | 59,93487 |
| Pig Mix 3 | 19,69264 | 5,42421 | 0,43858 | 63,73938 |
| Soy Mix 1 | 48,14465 | 6,64891 | 1,044 | 31,95878 |
| Soy Mix 2 | 52,90847 | 5,49428 | 0,42682 | 30,64225 |
| Soy Mix 3 | 52,25041 | 6,2094 | 0,71771 | 29,5788 |

Supplementary Table S7. Overview of both pathogen detection methods from all included meat and meat analogues, and all tested bacterial taxa. Culturomics are in CFU/100 μ L and metagenomics are in relative abundances (%). The metagenomics output are from the samples that were spiked with the bacterial cocktail as described in the main text. 1, 2 and 3 represent the 3 days of incubation, day 1, day 2 and day 3.

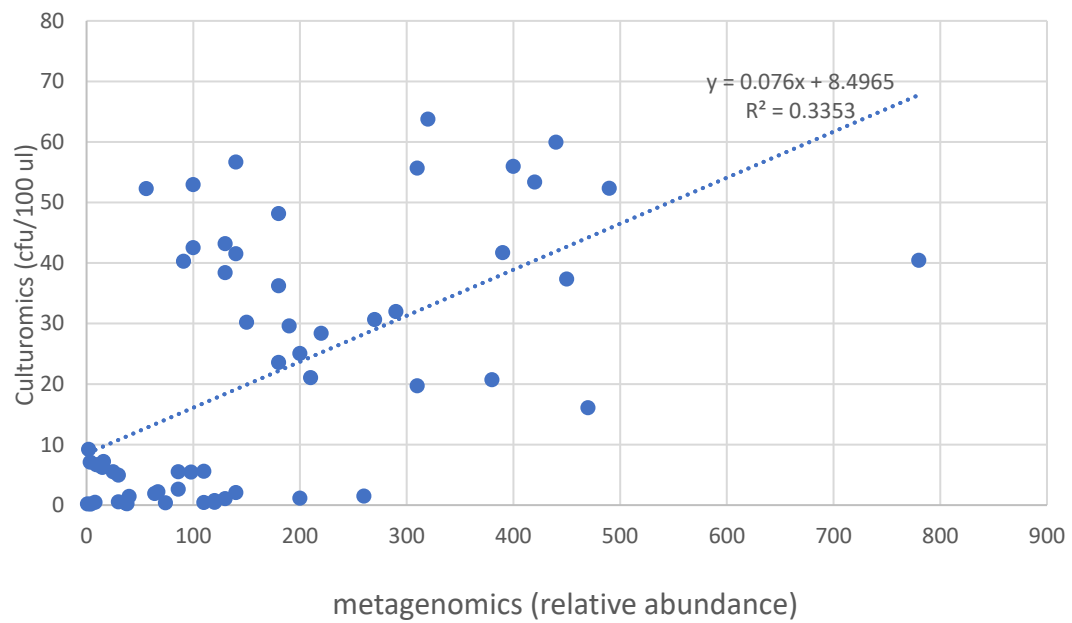
| | Chicken | | Beef | | Pig | | Soy | | Peas | |
|---------------|------------|--------------|------------|--------------|------------|--------------|------------|--------------|------------|--------------|
| | culturomic | metagenomics | culturomic | metagenomics | culturomic | metagenomics | culturomic | metagenomics | culturomic | metagenomics |
| E. coli 1 | 2 | 9,19263 | 30 | 4,92845 | 86 | 5,49055 | 9,3 | 6,64891 | 3,5 | 7,05854 |
| E. coli 2 | 1 | 0,1614 | 64 | 1,88599 | 110 | 5,57115 | 25 | 5,49428 | 13 | 6,46692 |
| E. coli 3 | 4 | 0,14638 | 260 | 1,4614 | 98 | 5,42421 | 15 | 6,2094 | 16 | 7,17402 |
| Listeria 1 | 110 | 0,40249 | 140 | 2,05032 | 200 | 1,11744 | 130 | 1,044 | 86 | 2,5974 |
| Listeria 2 | 38 | 0,1833 | 74 | 0,34616 | 30 | 0,51391 | 120 | 0,42682 | 40 | 1,40915 |
| Listeria 3 | 37 | 0,31021 | 37 | 0,3375 | 8 | 0,43858 | 120 | 0,71771 | 67 | 2,16702 |
| Cronobacter 1 | 470 | 16,07143 | 450 | 37,33244 | 150 | 30,19369 | 180 | 48,14465 | 130 | 43,17743 |
| Cronobacter 2 | 380 | 20,70064 | 220 | 28,36945 | 180 | 23,54396 | 100 | 52,90847 | 91 | 40,2535 |
| Cronobacter 3 | 210 | 21,02403 | 200 | 25,02477 | 310 | 19,69264 | 56 | 52,25041 | 100 | 42,50316 |
| Salmonella 1 | 780 | 40,41137 | 390 | 41,67876 | 490 | 52,31025 | 290 | 31,95878 | 180 | 36,20287 |
| Salmonella 2 | 400 | 55,94276 | 420 | 53,35242 | 440 | 59,93487 | 270 | 30,64225 | 140 | 41,49422 |
| Salmonella 3 | 140 | 56,65993 | 310 | 55,63213 | 320 | 63,73938 | 190 | 29,5788 | 130 | 38,37138 |



Supplementary Figure S1. Foodborne bacterial pathogen growth and survival over time in four meat and meat analogues using culture-dependent approach. Each bar represents the counted CFUs after the initial artificial inoculation date (day 0). Each number was adjusted to represent only the bacteria growth after adding the starting bacterial inoculum, and all CFU counts are adjusted to CFU/100 μ L volumes of the initial matrix-bacterial mix to be able to compare the outputs.



Supplementary Figure S2. Foodborne bacterial pathogen growth and survival over time in four meat and meat analogues using culture-dependent approach. Each bar represents the counted CFUs after the initial artificial inoculation date (day 0). Each number was adjusted to represent only the bacteria growth after adding the starting bacterial inoculum, and all CFU counts are adjusted to CFU/100 μ L volumes of the initial matrix-bacterial mix to be able to compare the outputs.



Supplementary Figure S3. Correlation between the detection levels of the foodborne pathogens using both culture-dependent methods (culturomics) and next generation metagenomic sequencing. With trend line and correlation R^2 values.