RESEARCH REPORT
PORK SAFETY

Title: Molecular Basis of Salmonella Competition in Broth Culture, **NPB #09-120**

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Date Submitted: March 1, 2011

Industry Summary:

Salmonella remains an extremely important bacterial pathogen to the swine industry. It is a significant pathogen affecting swine health and also represents one of the most important foodborne pathogens affecting people. A key aspect of *Salmonella* control is the use of cultivation methods in the laboratory. Samples must be cultured to isolate specific *Salmonella* strains, and this step is necessary to understand the spread of *Salmonella* throughout the swine production system. Unfortunately, conclusions about *Salmonella* transmission are highly dependent on the performance characteristics of these cultivation methods, and recently, we quantified a disturbing fact: the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample.

The overall objective of this project was to characterize the bias that cultivation media has on *Salmonella* detection and enumeration. To accomplish this, we conducted a series of competition experiments using *Salmonella* serovars and strains from the swine production system. We had the following specific aims:

1) Establish whether four *Salmonella* strains isolated from the swine production system exhibit heterogeneity in growth and competitive fitness during cultivation in broth media.
2) Determine which genes are either up or down regulated in these *Salmonella* strains in the presence of different cultivation broth media and during competition with other *Salmonella* strains.

To conduct our experiments, we used 4 different Salmonella e*nterica* serovars originally isolated from swine: *S.* Agona, *S.* Derby, *S.* Mbandaka, and *S.* Typhimurium. When grown individually in different media that are routinely used to culture *Salmonella* from swine samples, we found the following. In one broth, *S.* Derby exhibited the fastest growth and therefore appeared to have the potential to outcompete the other strains. However, in a second broth at 37°C, *S.* Derby did not grow at all. In this broth at 37°C, *S.* Typhimurium grew the fastest. In a third medium at 37°C, *S.* Agona grew the fastest. S. Derby was able to grow slightly in the early hours of the growth curve. When the serovars were grown in a Most Probable Number format, there were again major differences among strains. In general, the serovars had a more difficult time growing at 42°C than at 37°C, even though 42°C is often used. *S.* Derby again did not grow at all. These results began to demonstrate that important *Salmonella* serovars could be entirely missed using standard protocols.

When the strains were competed against each other in 2, 3 or 4-way competitions, there was a clear ordering of competitive ability among the strains, but this order depended on the media and temperature. Once again, *S.* Derby did not grow well and was never detected in any competition.

Finally, we identified specific genes in these strains that might be contributing to the differential growth characteristics. An understanding of the genetic basis of these differences could help us design a more appropriate cultivation protocol (with more appropriate media) to accurately culture all the *Salmonella* strains that might be present in swine samples.

This study confirmed what we had previously found in a pilot study: that the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample. We will now proceed to identify optimal strategies for cultivating *Salmonella* from swine samples.

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Keywords

Salmonella, competition, bias, diagnostics, cultivation media

Scientific Abstract

Salmonella remains an extremely important bacterial pathogen to the swine industry. It is a significant pathogen affecting swine health and also represents one of the most important foodborne pathogens affecting people. A key aspect of *Salmonella* control is the use of cultivation methods in the laboratory. Samples must be cultured to isolate specific *Salmonella* strains, and this step is necessary to understand the spread of *Salmonella* throughout the swine production system. Unfortunately, conclusions about *Salmonella* transmission are highly dependent on the performance characteristics of these cultivation methods, and recently, we quantified a disturbing fact: the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample.

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To conduct our experiments, we used 4 different Salmonella e*nterica* serovars originally isolated from swine: *S.* Agona, *S.* Derby, *S.* Mbandaka, and *S.* Typhimurium. When grown individually in different media that are

routinely used to culture *Salmonella* from swine samples, we found the following. In LB broth, *S.* Derby exhibited the fastest growth and therefore appeared to have the potential to outcompete the other strains. However, in R10 broth at 37°C, *S.* Derby did not grow at all. In this broth at 37°C, *S.* Typhimurium grew the fastest. In TTB at 37°C, *S.* Agona grew the fastest. *S.* Derby was able to grow slightly in the early hours of the growth curve.
When the serovars were grown in a Most Probable Number format, there were again major differences among strains. All serovars grew about equally well in LB at 37°C and 42°C. Differences in growth among serovars were seen in R10 and TTB at 37°C and 42°C. The serovars had a more difficult time growing at 42°C than at 37°C. Derby did not grow at all in TTB (APHA with Brilliant Green) at 37°C, TTB at 42°C (APHA with Brilliant Green), or in R10 at 42°C. Derby only survived in R10 at 37°C. Mbandaka grew better than Typhimurium in TTB. Typhimurium grew better in R10 than in TTB at 37°C and 42°C. Agona grew well in both R10 and TTB at 37°C and 42°C.

When the strains were competed against each other in 2, 3 or 4-way competitions, there was a clear ordering of competitive ability among the strains, but this order depended on the media and temperature. Once again, *S.* Derby did not grow well and was never detected in any competition.

Finally, we identified specific genes in these strains that might be contributing to the differential growth characteristics. An understanding of the genetic basis of these differences could help us design a more appropriate cultivation protocol (with more appropriate media) to accurately culture all the *Salmonella* strains that might be present in swine samples. The major genes of interest following this experiment were: cyoE (protoheme IX farnesyltransferase), cyoB (cytochrome o ubiquinol oxidase subunit), sdhC (succinate dehydrogenase C), sdhB (succinate dehydrogenase, FeS subunit), sucB (dihydrolipoamide succinyltransferase), cobT (nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase), and cbiE (cobalt-precorrin-6Y C(5)-methyltransferase).

This study confirmed what we had previously found in a pilot study: that the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample. We will now proceed to identify optimal strategies for cultivating Salmonella from swine samples.

Introduction

To design and implement *Salmonella* control strategies in the swine production system, it is imperative to have accurate data on the dynamics of *Salmonella* on the farm, during transport, and in the abattoir. If there is bias in the cultivation media that are commonly used, are we underestimating the importance of certain serovars or strains? As will be described in this proposal, we have found that there can be significant differences among *Salmonella* strains with respect to their growth characteristics in cultivation media. This study will quantify the disconcerting phenomenon that the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample. We hypothesize that there will be major differences in growth characteristics among some of the major *Salmonella* serovars / strains in the swine production system. Furthermore, we believe that it is possible to establish the molecular basis for this bias. This project will improve our understanding of the standard *Salmonella* cultivation media used in swine research and will help us develop more accurate inferences from our swine research studies, which, in turn, will lead to more effective *Salmonella* intervention programs.

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key aspect of *Salmonella* control is the use of cultivation methods. Samples must be cultured to isolate specific *Salmonella* strains, and this step is necessary to understand the spread of *Salmonella* throughout the swine production system. Unfortunately, conclusions about *Salmonella* transmission are highly dependent on the performance characteristics of these cultivation methods, and recently, we quantified a disturbing fact: the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample.

This project will help us better understand the bias that cultivation media impose on *Salmonella* studies within the swine production system. Through this project, which combines basic and applied science, we will gain valuable information into the effects that different broth media that are commonly used to culture *Salmonella* have on the accuracy of *Salmonella* cultivation and enumeration. We will elucidate whether this bias is due solely to the media or is exacerbated by competition among *Salmonella* strains when more than one strain is present in a sample. Finally, we will use current DNA microarray technology to examine the gene(s) that might be responsible for this bias.

This project will lead to a long-term research program with clear benefits to the swine industry. First, our research group believes that certain *Salmonella* strains / serovars are particularly well adapted to the swine production system. They thrive in the pig intestinal tract, in the pig environment, and possibly on the pig carcass as well. Our system for testing the bias associated with cultivation media is also perfectly suited for evaluating this swine ecosystem adaptation. We will be able to test whether certain *Salmonella* strains isolated from swine systems are more competitive that other non-swine *Salmonella* when cultured in swine-derived samples. By incorporating the DNA microarray in this type of analysis, we will identify genes that might be conferring this adaption to the swine environment. Whereas *Salmonella* vaccine targets are usually based on genes that provide an immune-related function, our analysis would provide a list of candidate genes that are functional in the process of adaptation to the swine environment but are not necessarily immunodominant.

Finally, this study and future work based on this study will enhance our ability to design effective *Salmonella* control programs, perform accurate *Salmonella* attribution analyses, and more confidently and accurately conduct *Salmonella* surveillance. Currently, we have no understanding of the performance characteristics of the cultivation media used in the swine production system for *Salmonella*. Some studies have assessed the sensitivity of the assays in different samples, but formal evaluations of inter-strain heterogeneity in growth characteristics in the media as well as the role of inter-strain competition in swine samples are lacking. This study will begin to develop a more comprehensive assessment of the performance characteristics and biases of *Salmonella* cultivation in the swine production system so that future research studies and intervention programs can be more accurate and effective.

Objectives

The overall objective of this proposal is to characterize the bias that cultivation media has on *Salmonella* detection and enumeration. To accomplish this, we will conduct a series of competition experiments using *Salmonella* serovars and strains from the swine production system. We have the following specific aims:

1) Establish whether four *Salmonella* strains isolated from the swine production system exhibit heterogeneity in growth and competitive fitness during cultivation in broth media.

2) Determine which genes are either up or down regulated in these *Salmonella* strains in the presence of different cultivation broth media and during competition with other *Salmonella* strains.

This study will quantify the disconcerting phenomenon that the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample.

Materials and Methods

For this project, 4 serovars of *Salmonella enterica* subsp. *enterica* originally isolated from swine were used. These strains were:
Salmonella enterica subsp. *enterica* Agona (*S*. Agona)
Salmonella enterica subsp. *enterica* Derby (*S*. Derby)
Salmonella enterica subsp. *enterica* Mbandaka (*S*. Mbandaka)
Salmonella enterica subsp. *enterica* Typhimurium (*S*. Typhimurium)

Growth Curves

Prior to conducting competitions among the strains, it was important to ascertain how these strains grow in different culture media when inoculated as pure strains. Each strain was grown in a static overnight culture in buffered peptone water (BPW) at 37°C. Cultures were diluted 1:2 in BPW and then diluted 1:100 into LB, R10 and TT broths. Multiple replicate 3 ml cultures were set up in tubes and incubated at 37°C in a water bath. The 0-hour time points for each serovar were staggered about 20 minutes apart to allow adequate time for plating. Growth was monitored every hour by plating a dilution from each culture tube onto one LB agar plate. LB agar plates were incubated at 37°C overnight. Colonies on the LB agar plates were then counted, and cell concentrations for each serovar were calculated using the colony counts.

Most Probable Number (MPN)

An MPN series was set up for each strain to see whether the same inoculum would generate the same growth pattern (and thus the same predicted cells/ml) in LB, R10, and TTB (APHA with Brilliant Green). Cultures were grown overnight in BPW at 37°C. The cultures' starting concentrations were measured by O.D. 600 readings and were equilibrated by adding BPW. The concentrations were assumed to be about 10^9 cells per ml. Cultures were diluted serially 10-fold in BPW to an estimated 10^5 cells per ml. The fifth and sixth 10-fold dilutions were made in the MPN media (LB, R10, or APHA TTB). The series was performed in culture blocks

using a 3-tube MPN format. The MPN blocks contained 800 µl medium per well. The first row of the MPN contained 800 µl medium and 200 µl of the sixth 10-fold dilution. From the first row, seven 5-fold dilutions were made down the row of wells. 200 µl medium was removed from the last row. MPNs were performed in triplicate. Six MPN blocks were prepared: LB, R10, and TTB media, each at 37°C and 42°C. Each block contained the four serovars in one of the media. After 24-hour incubation, 1µl from each MPN well was spotted onto XLT4 plates to check for growth. Plates were incubated at 37°C.

Salmonella Competitions

To determine the growth characteristics of the different *Salmonella* strains when competing against other strains in culture, a series of competition experiments were performed. Cultures were grown overnight in buffered peptone water (BPW) at 37°C. Concentrations were then normalized by adjusting the optical density A600 values of the overnight cultures. Plate counts conducted simultaneously confirmed that the A600 values were in agreement with CFU/ml values. Three 10-fold dilutions in BPW were done for each culture.

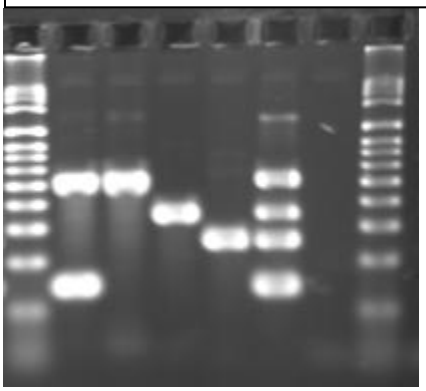
Competitions were performed in R10 or TTB at 42°C or 37°C. For the four-way competitions, the ratio of serovars was 25:25:25:25. For the three-way competitions, the ratio of serovars was 33:33:33. For the two-way competitions, the ratios of serovars were: 10:90, 90:10, and 50:50. For each ratio, three concentrations were tested: 10,000 cells per ml, 1,000 cells per ml, and 100 cells per ml. Culture tubes were incubated in a water bath at 42°C or 37°C. After 24 hours of growth, the cultures were diluted in PBS and plated onto XLT4 agar. Plates were incubated at 37°C. The following day, 48 colonies from each XLT4 plate were picked and spread onto LB agar plates so that the colonies could be saved for typing. To avoid biased picking, all colonies from one area of the plate were picked. Plates were incubated at 37°C.

To determine the strain of each colony that was saved, a small portion of each colony was transferred to a PCR well containing 30 µl sterile dH_2O. This mixture was boiled for 10 minutes to lyse the cells and then centrifuged. The supernatant was used as the template for subsequent PCR reactions. Each PCR well contained 12.5 µl. Primer mix consisted of equal amounts of all forward and reverse primers for each serovar (8 primers total). dNTP mixture contained equal amounts of each dNTP. TetKLM was the PCR program used. PCR products were separated in 1.5% agarose gel to type the colonies.

PCR reaction (98X):

- 5X green buffer: 245.0 µl
- 24 µM primer mix: 70.0 µl
- 25 mM $MgCl_2$: 122.5 µl
- 100 mM dNTPs: 12.25 µl
- Template: 98.0 µl
- Taq: 7.35 µl
- Sterile dH_2O 670.0 µl

Figure 1: Gel image based on multiplex PCR for *Salmonella* strain identification.



Lanes 1 and 8: 100 bp QuantiMarker
Lane 2: *S.* Agona
Lane 3: *S.* Derby
Lane 4: *S.* Mbandaka
Lane 5: *S.* Typhimurium
Lane 6: Mix of 4 strains, multiplex of 4 primer sets
Lane 7: Negative control

Primers for the multiplex PCR was as follows:
Salmonella B serovar (ForwardG/ReverseG, 531 bp), Herrera-Leon 2004, J. Clin Microb. 42:2581
Salmonella C serovar (rfbC1-L/rfbC1-R2, 340 bp) designed by our laboratory using Primer3
Salmonella phase I i serovar (Sense60/Antisense-i, 253 bp) Herrera-Leon 2004, J. Clin Microb. 42:2581
Salmonella Agona specific region of *rfb* gene (Agona-L/Agona-R1, 137 bp) designed by our laboratory using Primer3

All four primer sets could be multiplexed, so each colony only needed one PCR reaction to identify the strain.

Presence of a soluble factor

We wanted to determine whether specific *Salmonella* strains secrete factors that inhibit competitors, thereby explaining the competition phenomenon when multiple strains are grown together, as well as whether physical contact is necessary for the winner/loser of a competition. In other words, if there is a factor secreted by a strain that inhibits competitors, is this factor soluble or does it require cellular contact.

We used 12-well plates (BD product 353503) fitted with 0.4 micron inserts (BD product 353494). First, we checked whether *Salmonella* can get through the filter. We then grew strains in a 2-way fashion, with one strain

on each side of the filter insert. We replicated each of these growth trials three times. Each triplicate trial was then duplicated by reversing the side of the insert that each strain was placed.

Differential Gene Expression Using Microarray Analysis

The purpose of the microarray analysis was to compare the expression patterns of the different *Salmonella* strains in 3 different media to help determine why one strain can out-compete the other in a particular medium. Specifically, we wanted to determine if there is a specific set of genes that might be involved in providing a competitive advantage to one strain versus another.

Each strain was grown in LB, in R10, or in TTB to early/mid log phase. This allowed us to find the time point when the cells were actively growing but were only at 25-30% of the cell concentration where the growth curve would reach an asymptote. Cell growth was stopped quickly by chilling, centrifuging, resuspending in a small volume of Davis minimal medium, then adding an equal volume of RNAlater. RNA extraction, labeling, and hybridization were done with some modifications to the methods received from the McClelland lab, but all were done by a uniform procedure so any processing artifacts would be the same between all.

The array slides are described at
http://dh.sdibr.org/MMCC_ORF_array_datasheet_090615b.pdf

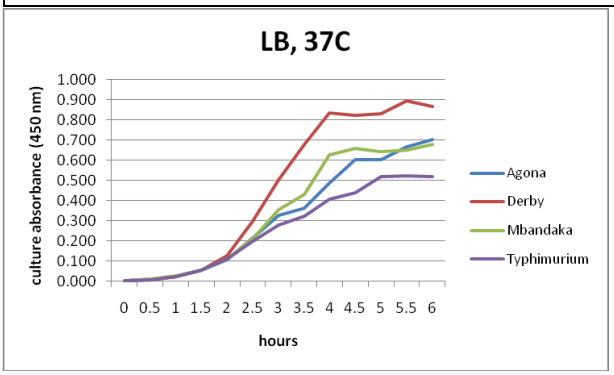
Web array analysis is described at
http://www.webarray.org/webarray/index.html

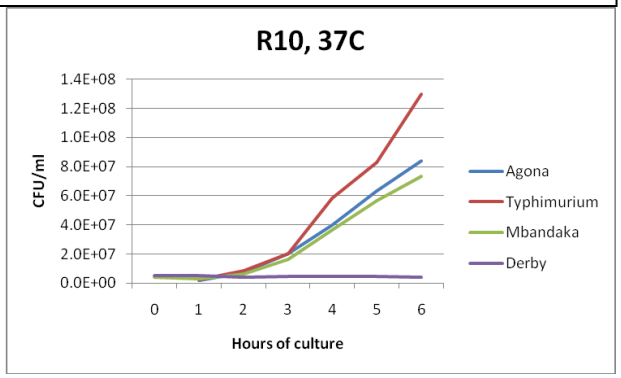
Results

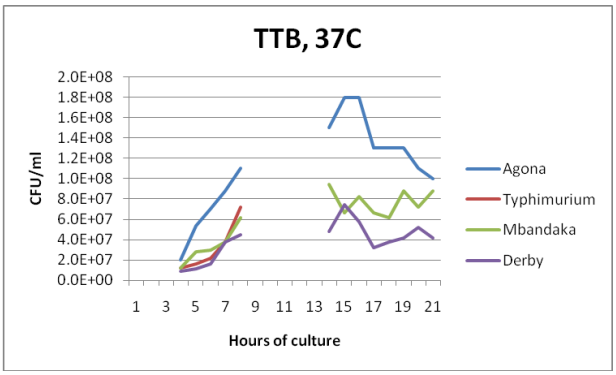
Growth Curves

In LB, *S.* Derby exhibited the fastest growth and therefore appeared to have the potential to outcompete the other strains. However, in R10 at 37°C, *S.* Derby did not grow at all. In this broth at 37°C, *S.* Typhimurium grew the fastest, which is in contrast to how it grew in LB at the same temperature. In TTB at 37°C, *S.* Agona grew the fastest. *S.* Derby was able to grow slightly in the early hours of the growth curve.

Figure 2: Growth curves of the four strains in three different media at 37C.







Most Probable Number (MPN)

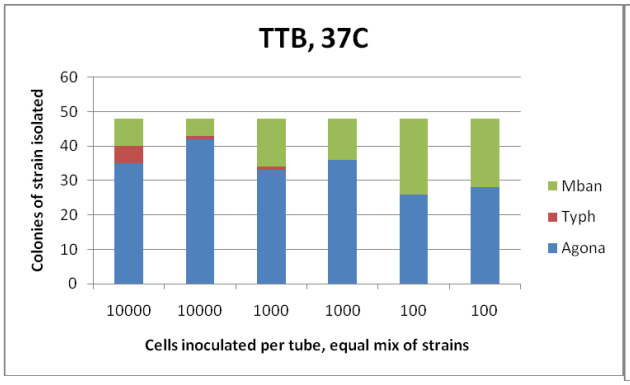
All serovars grow about equally well in LB at 37°C and 42°C. Differences in growth among serovars are seen in R10 and TTB at 37°C and 42°C. The serovars have a more difficult time growing at 42°C than at 37°C. *S.* Derby does not grow at all in TTB (APHA with Brilliant Green) at 37°C, TTB at 42°C (APHA with Brilliant Green), or in R10 at 42°C. *S.* Derby only survives in R10 at 37°C. *S.* Mbandaka grows better than *S.* Typhimurium in TTB. *S.* Typhimurium grows better in R10 than in TTB at 37°C and 42°C. *S.* Agona grows well in both R10 and TTB at 37°C and 42°C.

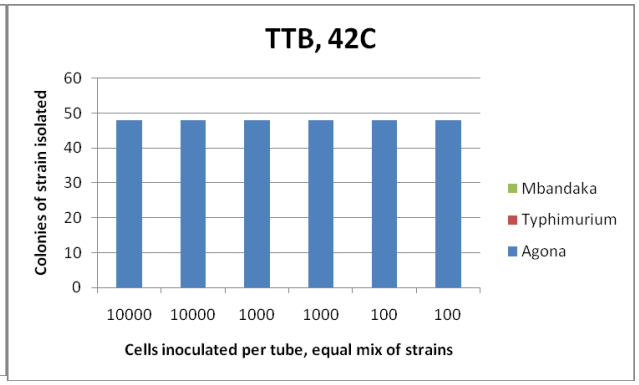
MPNs can somewhat predict which serovar will win in competitions. MPNs appear to correlate with the 4-way competition results. From the MPN data, we predicted which 2-way competitions would be insightful.

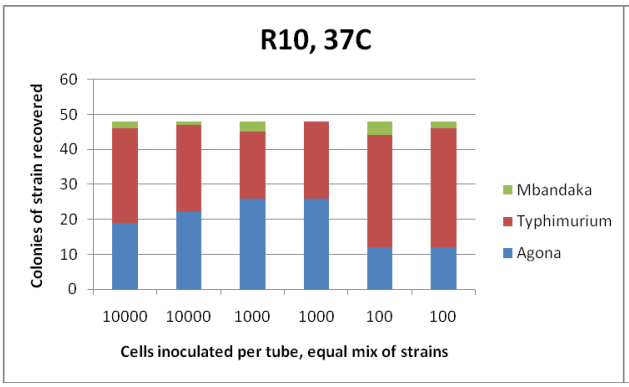
Salmonella Competitions

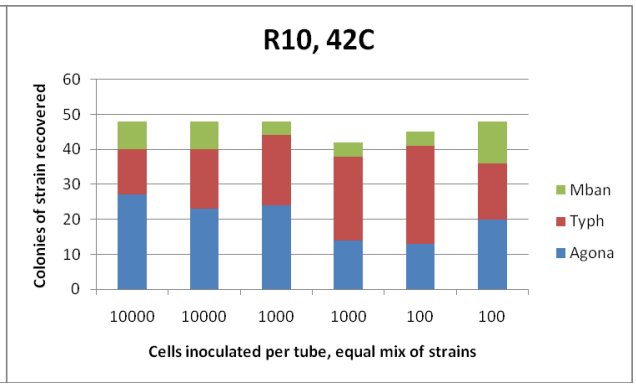
The 2-way competition results were mirrored in the 3-way and 4-way competition results. *S.* Agona and *S.* Typhimurium are the stronger competitors in R10. *S.* Typhimurium does slightly better than *S.* Agona in R10 at 37°C. *S.* Agona does slightly better than *S.* Typhimurium in R10 at 42°C. At 37°C in TTB, *S.* Agona is the strongest competitor. *S.* Mbandaka grows better than *S.* Typhimurium in TTB at 37°C. At 42°C in TTB, only *S.* Agona colonies were found. *S.* Derby does not grow well in R10 or TTB; it was never detected in any competition. *S.* Typhimurium does not grow well in TTB that contains Brilliant Green, especially at 42°C. Interestingly, the addition of Brilliant Green to TTB is standard protocol.

Figure 3: Results of 3-way competitions in different media at different temperatures. The figures shown below represent the competitions in which all three strains were competed at equal starting concentrations.









Presence of a soluble factor

The experiments using the culture inserts showed no evidence of a soluble competition factor; strains of disparate competitive ability grow to the same final concentrations when they are on opposite sides of a semi-permeable membrane. Strains separated by a culture well insert are unaffected by the strain on the other side. We therefore decided to skip the microarray analysis on cells from insert-separated wells.

Regarding the question of whether physical contact is necessary to win competition, we were unable to answer this question with the culture inserts. If the main reason for competition success is the intrinsic growth rate and saturation concentration of each strain in each medium, then physical contact is not necessary.

Differential Gene Expression Using Microarray Analysis

An example of the kinds of analyses that can be made is shown in figure "TTB heatmap". All strains show up-regulation of the *cyo*, *sdh*, and *suc* gene families when grown in TTB compared to when grown in LB. In contrast, *S.* Agona shows up-regulation in the *cob* and *cbi* gene families, while the other strains are down-regulated; these genes could be investigated to see whether they provide *S.* Agona with a competitive advantage in TTB. At least 8 other regions of the 4600+ open-reading frames show expression differences could that warrant further investigation.

cyoE: protoheme IX farnesyltransferase
cyoB: cytochrome o ubiquinol oxidase subunit
sdhC: succinate dehydrogenase C
sdhB: succinate dehydrogenase, FeS subunit
sucB: dihydrolipoamide succinyltransferase
cobT: nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase
cbiE: cobalt-precorrin-6Y C(5)-methyltransferase

Figure 4: Heat maps of the 4 strains showing genes that are up or down regulated in the presence of TTB.

Gene	Typhimu	Derby	Mbanda	Agona	4 serovars (average)
	0.060881	-0.16076	-0.17859	-0.25602	-0.12941
	0.242951	-0.48905	-1.32397	-1.05658	-0.62051
	0.724291	0.486895	0.349256	0.180259	0.426411
cyoE	3.793808	3.704417	3.958849	4.517791	3.949094
cyoD	2.957467	2.902364	4.097748	4.273928	3.481599
cyoC	2.920993	2.985591	3.88619	4.121746	3.405681
cyoB	3.390033	3.111459	3.671259	4.114917	3.508073
cyoA	3.251666	3.805568	2.724364	3.924618	3.375588
ampG	0.202695	-0.402	0.552937	0.465385	0.220347
yajG	-0.72173	-1.05427	-0.35881	-0.49649	-0.63892
bolA	-0.61323	0.951058	0.28418	0.243463	0.235504
tig	-0.61446	-0.56553	-0.51013	-1.07524	-0.69855

	-0.17449	0.761167	-0.50501	-0.63926	-0.12085
nei	0.594638	0.21948	-0.00536	0.75883	0.38393
abrB	0.923401	1.033224	0.837471	0.982747	0.915071
gltA	2.957447	5.398034	3.611866	3.860409	3.91045
	2.183917	4.22406	3.167321	2.934393	3.071936
sdhC	3.666655	5.416016	4.162042	4.756456	4.407936
sdhD	3.593001	5.051075	3.882161	4.608776	4.212413
sdhA	3.680247	5.400837	4.231789	5.353733	4.62341
sdhB	2.022633	3.235968	4.127411	4.583838	3.440998
sucA	1.817628	5.031267	3.806192	5.006943	3.803435
sucB	1.218683	4.456294	3.119472	4.817621	3.344065
sucC	1.39476	4.438282	3.661234	4.153604	3.323306
sucD	0.749095	3.965931	4.018085	3.998545	3.121858
cydA	0.03444	0.427913	0.333425	1.294581	0.504728
cydB	0.233913	1.082066	0.605094	1.66478	0.876563
ybgT	0.406509	0.414814	0.246829	0.772297	0.461369
ybgE	0.956104	0.85521	1.130635	1.239223	1.038286
yeeO	-0.04657	0.444952	0.009121	0.86323	0.293812
erfK	-0.51228	0.482093	-0.14528	2.225832	0.497251
cobT	-0.56909	0.4782	-0.4269	2.28621	0.429643
cobS	-0.65767	0.742208	-0.55315	1.947667	0.340497
cobU	-0.89339	0.150696	-0.45299	1.739589	0.122751
cbiP	-0.95556	0.638648	-0.67769	2.601061	0.372501
cbiO	-1.58683	0.027025	-1.54759	1.769408	-0.36112
cboQ	-1.50716	0.700151	-1.13351	2.146694	0.033901
cbiN	-1.95252	-0.12352	-1.70187	1.585029	-0.57107
cbiM	-1.65045	0.356569	-1.17452	2.274496	-0.06489
cbiL	-1.52423	0.324687	-1.57191	1.946099	-0.22863
cbiK	-2.08257	0.200104	-1.8627	1.551122	-0.58277
cbiJ	-1.86701	0.423105	-1.67959	2.237083	-0.25135
cbiH	-1.79718	0.003297	-1.13502	2.223451	-0.20109
cbiG	-1.82387	0.251672	-1.51091	2.252958	-0.23714
cbiF	-1.42025	-0.3081	-1.504	1.822136	-0.37597
cbiT	-2.30399	-0.40679	-1.85357	1.646013	-0.74698
cbiE	-2.0268	-0.57226	-2.38049	2.076593	-0.76277
cbiD	-2.12532	-0.80923	-1.58749	1.926595	-0.66547
cbiC	-1.90271	-0.73103	-1.60661	2.783393	-0.3889
cibB	-1.82689	-1.13625	-1.62769	2.165881	-0.62057
cbiA	-1.97067	-1.05824	-1.77987	1.592706	-0.82105
pocR	-1.44589	-0.72093	-0.71111	0.996424	-0.49115
pduF	0.014648	0.289961	0.225714	1.139452	0.381414

Discussion

This study confirmed what we had previously found in a pilot study: that the probability of detecting a specific *Salmonella* strain in a sample might have very little to do with its concentration in the sample but more to do with its ability to compete in the cultivation media and with the specific mixture of *Salmonella* strains present in the sample. Cultivation protocols that are standards for detecting and enumerating *Salmonella* from swine samples would fail to culture key strains, such as *S*. Derby. While this possibility has been recognized previously, the cultivation protocols have not been altered to reflect this potential. In competition, there was a clear ordering of strains, and therefore, if multiple strains were present on a swine farm, this diversity might go undetected. This has major implications for *Salmonella* control strategies and surveillance.

We identified genes that were up-regulated in the most competitive strains, and these genes could therefore be responsible for helping with the competitive advantage. The knowledge of which genes are up-regulated could be used in a couple of ways. First, it might enable the development of cultivation protocols that maximize the likelihood of culturing all strains that are present in the sample. Second, the evaluation of these genes in the presence of swine material could indicate their role in adaptation to the swine environment.