RESEARCH ABSTRACT

PORK SAFETY

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

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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.

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 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.