RESEARCH REPORT
SWINE HEALTH

Title: *Haemophilus parasuis* Proteomics for Vaccine Design, NPB #01-141

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II Objectives:

1. Compare the proteomics of *H. parasuis* serovars that cause disease with death (serovars 1, 5, 10, 12, 13, and 14), disease without death (serovars, 2, 4, 8, 15), and serovars that are avirulent (serovars 3, 6, 7, 9, and 11).
2. Determine optimal conditions that result in altered two-dimensional protein patterns upon iron limitation.
3. Identify newly expressed proteins from immunoblots of two-dimensional gels by using sera from infected, convalescent, and vaccinated swine for the development of improved vaccines.

III Progress toward meeting objectives

Objective 1. We have compared the virulent serovars versus the avirulent serovars using comparitive proteomics. Relative levels of protein expression were compared. Proteins unique to virulent serovars and those unique to avirulent serovars were identified.

Objective 2. Determination of protein expression as a result of iron limitation was difficult to perform as each batch of medium was differed in its iron composition. We concentrated instead on the comparisons of the serovars for selecting proteins of interest.

Objective 3. Immunoblots were not performed. Instead, we selected and identified two unique and two common proteins for further work.

IV Status of project in regards to stated timeline

We have completed the objectives and identified proteins of interest for further study.

V Modifications of project from original proposal

We changed the direction of Objectives 2 and 3, when we found that the medium composition varied due to inclusion of horse serum that is necessary to obtain adequate growth. Instead we proceeded with the systematic comparison of the 2-D profiles of the expressed proteins for the identification of potential target proteins for vaccine design.

VI Results

Progress summary. Since the last report, we have compared in detail the 2-D protein profiles of virulent and avirulent serovars. In order to proceed with the comparison, a composite 2-D profile was prepared. This composite profile allows the direct comparison of protein expression of virulent and avirulent serovars. An example of such a comparison is shown in Fig. 1. This comparison shows proteins that are in common between two sets of avirulent serovars that are not seen in the virulent serovars (spots 1 through 4). Similarly, spots 5 through 12 are proteins in common with two sets of virulent serovars that are not present in the avirulent serovars.

Fig. 2 shows proteins spots that were selected for further analysis by mass spectrometry. The proteins selected were two common proteins that were differentially expressed, and two unique proteins, one for the virulent serovars and one for the avirulent serovars. Differential expression of the proteins is shown in Table 1. Protein expression by the virulent serovars for spot 5502 and 7501 are three- and two-fold, respectively, for those estimated for the avirulent serovars.

Table 1. Differential protein expression of four proteins expressed by virulent and avirulent serovars

Spot number, SSP	Virulent *H. parasuis* 1 & 10	Avirulent *H. parasuis* 3 & 9
	Relative density $\pm$ CV	Relative density $\pm$ CV
5502	9380.4 $\pm$ 78.9	2971 .5 $\pm$ 28.3
6601	5327.3 $\pm$ 20.1	none
7501	4711.6 $\pm$ 81.1	2381.7 $\pm$ 48.1
8403	none	2739.8 $\pm$ 38.7

The proteins were identified by peptide mass fingerprinting, mass spectrometry and database mining. Table 2 shows the protein homologies rather than identities because the genome for *H. parasuis* has not yet been sequenced

Table 2. Protein homologies

Spot number	MOWSE score	Masses matched	% Coverage of sequence	MW/pI	Accession #
5502	9.9	9/17 (50%)	50%	88,664/6.6	14586745
6601	6.64	4/20 (20%)	7%	87,512/7.6	032629
7501	26.0	4/12 (33%)	53%	36,328/6.2	P45095
8403	7.7	5/8 (62%)	56%	39619/9.3	Q48216

5502-homologous to integral outer membrane protein of *Haemophilus ducreyi*
6601-homologous to protective surface antigen precursor D15 of *H. influenzae*
7501-homologous to dipeptide transport ATP-binding protein, dppD, of *H. influenzae*
8403-homologous to outer membrane protein P2 of *H. haemophilus*

In summary, we have compared the 2-D protein profiles of two virulent and two avirulent serovars and identified both unique and common proteins. Three of the proteins for which homologies were identified are associated with the outer membrane. One protein in particular is of interest, the protective antigen precursor protein that belongs to the D15 family of proteins of *H. influenzae*. We plan to clone the gene encoding this protein using primer sequences based on the *H. influenzae* gene sequence for the D15 protein.

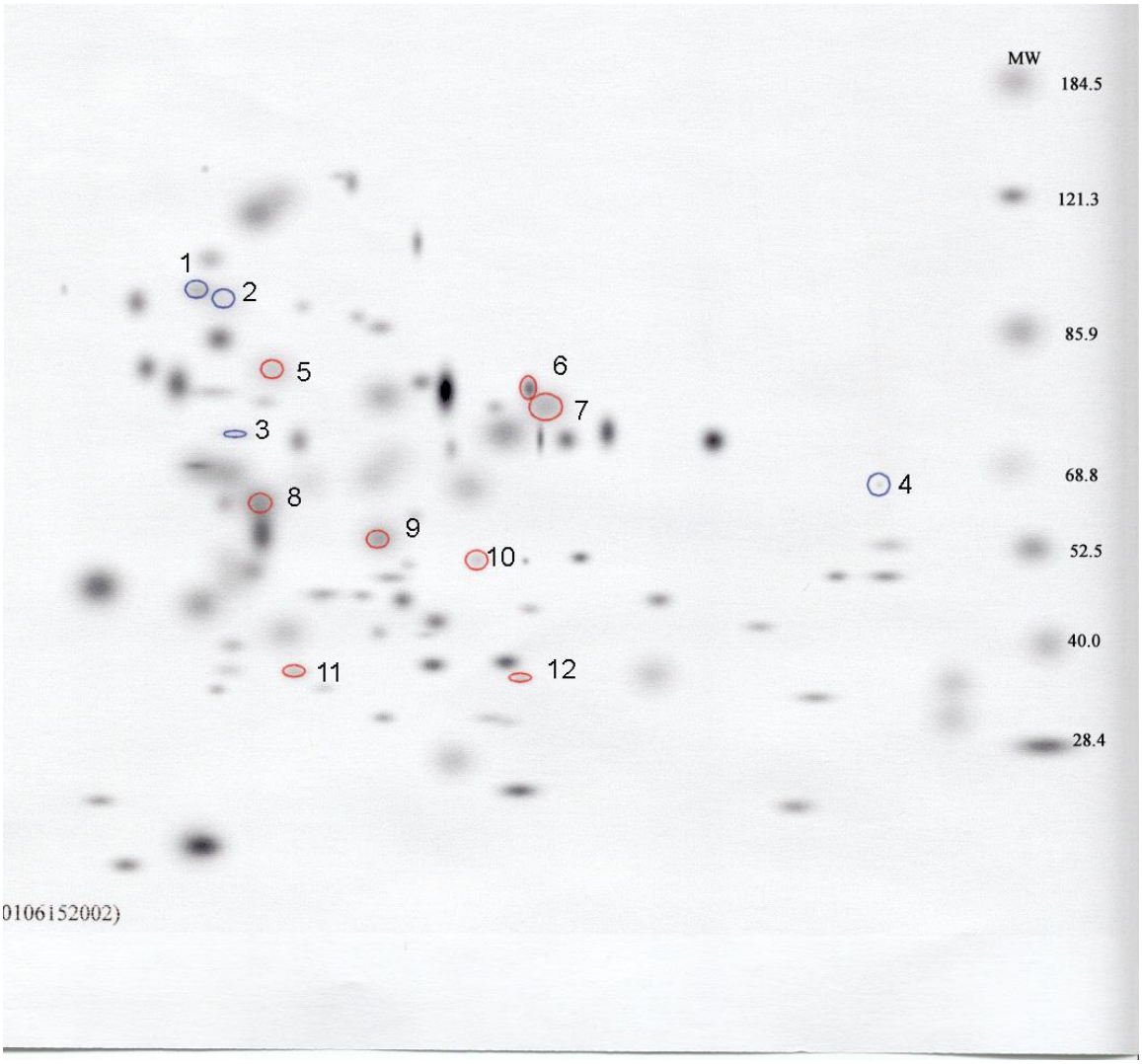

Fig. 1. Composite 2-D gel of expressed proteins from avirulent and virulent *H. parasuis* serovars. Spots marked 1 through 4 are expressed proteins present in avirulent serovars 3 and 9 that are not present in the virulent serovars. Spots marked 5 through 12 are expressed proteins present in virulent serovars 1 and 10 that are not present in avirulent serovars.

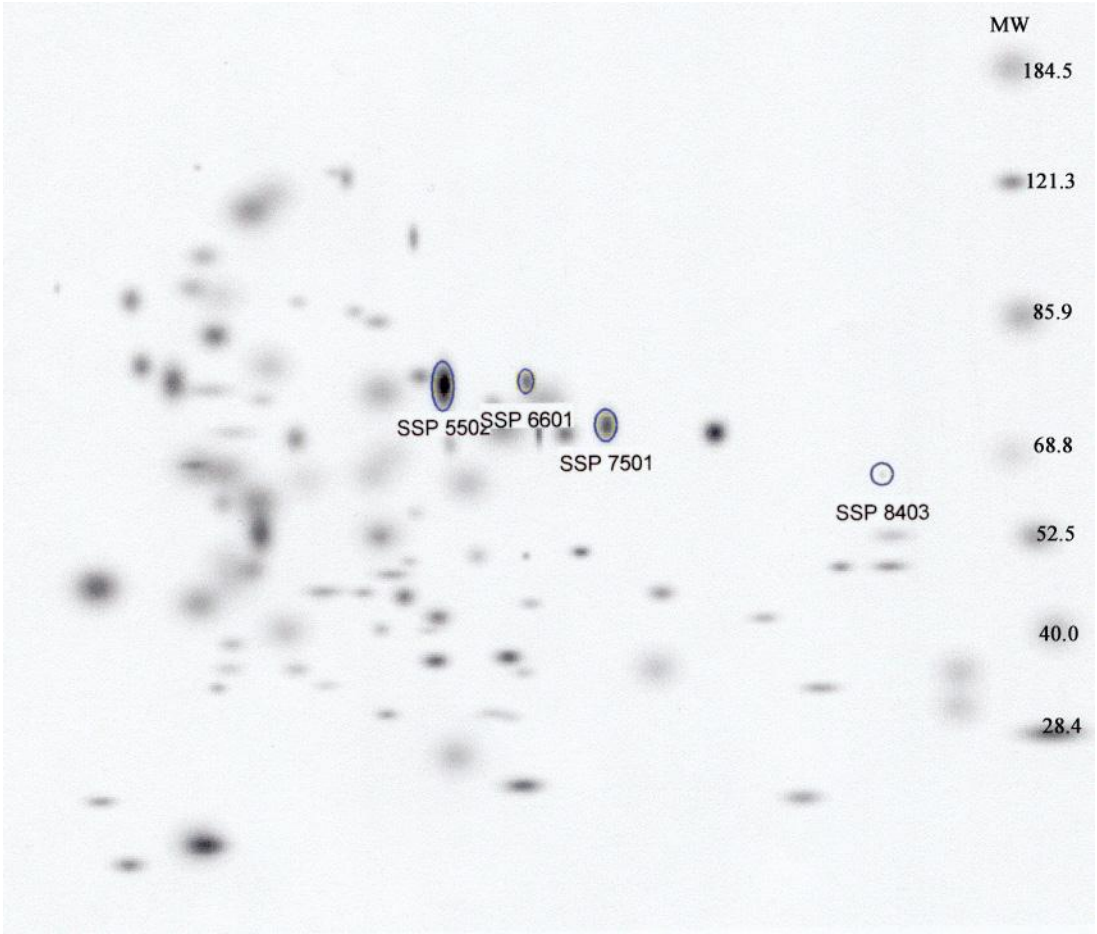

Fig. 2. Mass spectrometric analysis of protein spots. SSP 5502 and 7501 are present in both virulent and avirulent serovars of *H. parasuis*. SSP6601 is unique to virulent serovars and SSP 8403 is unique to avirulent serovars. See Table 1 for protein identification.