# Protein Profiling in *Wangiella dermatitidis*

By

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**Abstract**

*Wangiella dermatitdis*, a dematiaceous fungus, exhibits three distinct morphologies: monliform or true hyphae, unicellular budding yeasts, or multicellular forms. This study was set up to establish a proteomic protein profile of the wild type form of *W. dermatitidis*. It is hypothesized that there will be significant differences in protein expression between the yeast grown at the permissive temperature of 25°C and restrictive temperature of 37°C.

Yeast cultures of *W. dermatitidis* were cycled and incubated for 48 hours at 25°C and 37°C before harvesting. The yeast cells were isolated using Tri Reagent and sonication. The proteins were then separated using two-dimensional gel electrophoresis and Coomassie Blue staining. Proteins similarities and differences were analyzed using PDQuest imaging software. The significant differences that were found could lead to identification of the proteins that may play a role in the virulence factor of *Wangiella dermatitdis*.

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**Acknowledgements**

There have been many people that I have met along the way here at Youngstown State University who have helped me with my trials and tribulations.

I would like to begin by thanking my friends, Tammy, Christine, Doug and Jenny. You allowed me to call you day or night and listen to my many frustrated stories of proteomics. I greatly appreciate all of your patience, laughter, advise and value our friendships. Doug, thank you for tolerating my frustration, even when they weren't always fair to you. I hope I can offer you the same help while you complete your masters. I wish you all the best in your travels.

Thank you to Pat Sudzina, Lisa, Tom, Heather and Don for enduring my questions. I know they seemed redundant and confused, but I like to be sure of things. Jamie and Lisa, I appreciate you checking my thesis for errors and incompetencies. I know this wasn't fun and was tedious, but you both deserve medals for your help.

To my family, who at times, thought I might be building biological weapons at school. Thank you for not asking me five hundred questions and for taking care of my babies while I spent many nights away at school. Bailey and Bart, thank you for making life fun, cheerful and interesting and for your unquestionable loving, loyalty.

Thank you to my committee, Dr. Walker, Dr. Asch and my advisor, Dr. Cooper, for helping me along the way. I came into this project with a brief knowledge of proteomics and walked out with a wealth of knowledge. I appreciate what you have given me and for spending the time with me. I wish you all the best.

Finally, thank you God for helping me with my results. I knew if I talked enough they would come! And who said talking to your project wouldn't help?

## Introduction

The dematiaceous fungus *Wangiella dermatitidis,* which belongs to the class Fungi Imperfecti, has been an area of interest for years due to its exhibition of polymorphism. Polymorphism enables *W. dermatitidis* to take on three very distinct morphologies: moniliform or true hyphae, unicellular budding yeasts, or multicellular forms (Figure 1). Each of these forms has very individualistic properties and structures. Also, *W. dermatitidis* is an excellent model for study due to its polarized growth and cellular differentiation, as well as the existence of cell-wall related virulence factors (Szaniszlo and Momany 1993; Szaniszlo et al. 1993) As an asexual fungal pathogen, *Wangiella dermatitidis* has been associated with primary dermatotrophic forms of cutaneous and subcutaneous phaeohyphomycosis (Kwon-Chung and Bennett, 1992). Hence, *W. dermatitidis* can serve as a model for other infectious dermatiaceous fungi.

### Polymorphism in *Wangiella dermatitidis*

The yeast cell cycle has four phases: $G_1$, S, $G_2$ and M. The S phase is the period of DNA synthesis. The $G_2$ phase is usually where bud emergence occurs and M phase is the period of mitosis. Bud emergence is not dependent on DNA synthesis (Roberts and Szaniszlo, 1980).

The moniliform or true hyphae form is characterized by apical extension and by budding growth associated with the production of blastoconidia and phialo- or annelloconidia (Grove et al., 1973; Oujezdsky et al., 1973; Cole, 1978; Hironaga et al., 1981). Hyphal buds arise from inner layers of the cell wall usually by blastic growth. The conidia arise from the apices of undifferentiated conidiophore that are derived from

hyphal branches or are made directly from the hyphal wall (Oujezdsky et al., 1973; Cole, 1978).

*Wangiella dermatitidis* develops the unicellular form by budding (Figure 3). During this process, the yeast cells form a bud, usually near the poles of the ellipsoidal cell, which will expand to approximately two thirds the size of the original cell. The yeast cells and buds are uninucleated. At this time, the nucleus of the mother cell will migrate to the bridge that is formed between it and the daughter cells. Next, mitosis occurs followed by cell separation, leaving a new daughter cell and the previous mother cell. The new yeast cell that is formed usually has a thin wall (Grove et al., 1973; Roberts et al., 1979; Jacobs and Szaniszlo, 1982).

The multicellular form occurs in response to inhibition of the cell-cycle event of bud emergence without the inhibition of cellular growth, nuclear division or cytokinesis (Szaniszlo et al., 1976; Roberts and Szaniszlo, 1978, 1980; Roberts et al., 1979). The multicellular form found in the mutant strain Mc3 is characterized by a switch from a non-polarized istropic form to the multicellular, multinucleated form. The multicellular form often times resembles sclerotic bodies. Sclerotic bodies are isotropically enlarged cells containing a septum. Multicellular form development can be induced by growing the yeast in a very acidic medium or long-term incubation on solid medium (Szaniszlo et al., 1976). The conversion of *W. dermatitidis* from the yeast form to the multicellular form consists of two phases: Stage I and Stage II.

Stage I of the conversion creates non-septate, swollen, unbudded cells with many nuclei and thickened cell walls. This stage can be stimulated in various ways. First, the

wild type yeast strain may be incubated on a solid medium for two to four weeks producing large quantities of stage I cells (Oujezdsky et al., 1973). Second, incubating the wild type in acidic medium allows for Stage I to occur within a few days (Szaniszlo et al., 1976). Third, the multicellular mutant strains Mc2 and Mc3 can be incubated at 37°C and stage I development occurs in one cellular cycle or in about five and a half hours (Jacobs and Szaniszlo, 1985). Stage II is a continuation of stage I growth. During stage II, the isotropic growth continues forming one or more internal, transverse septa, creating the multicellular phenotypes (Cooper and Szaniszlo 1993).

The cell wall of *W. dermatitidis* consists of large quantities of chitin. The multicellular forms contain 10 times the amount of cell wall chitin than yeast cells grown at 25°C and six times more than yeast grown at 37°C (Geis, 1981). If chitin synthesis is inhibited during the multicellular form development, it causes cell lysis. This suggests that chitin plays a role in cellular stabilization during the developmental process (Geis, 1981; Cooper et al., 1984). Polyoxins are used to inhibit chitin synthesis (Endo et al., 1970). This characteristic of polyoxin can be used to prevent the conversion to the multicellular form in stage I, which induces cell lysis (Geis 1981; Cooper et al., 1984). In yeast cells, polyoxin inhibits cell separation and causes the formation of chains or clusters of cells that have irregular septa (Szaniszlo et al., 1983; Cooper et al., 1984). When the Mc3 mutant is grown at 37°C, the collective composition of the cell wall drastically changes. There are drastic changes in the quantity of melanin, *N*-acetyl-glucosamine, ash and sugar (Geis, 1981). It has been shown that both the wild type and Mc3 strains do not require melanin for yeast growth or for multicellular development (Geis, 1981).

Polymorphism is also regulated by intracellular calcium. When all other conditions were constant, lower concentrations of calcium favored non-polarized growth that would eventually lead to the multicellular form. Elevated concentrations of calcium ions, promoted polarized growth. Polarized growth is characterized by yeast budding or true hyphal growth. Calcium-ion-dependent processes at 37°C include isotropic growth, mitosis, septation, polarized yeast budding, and hyphal apical extension are all calcium ion dependent processes (Karuppayil and Szaniszlo 1997).

**Goals**

My objectives for this study are to characterize the proteomic protein profiles in the wild-type strain of *Wangiella dermatitidis*. The profiles will include the permissive temperature of 25°C and the restrictive temperature of 37°C. All research trials will be done in triplicate to ensure results. This research should be able to give others insight into some of the characteristics of other yeasts with polymorphic tendencies.

**Figure 1.**

Transitions among the vegetative morphologies of *Wangiella dermatitidis.*

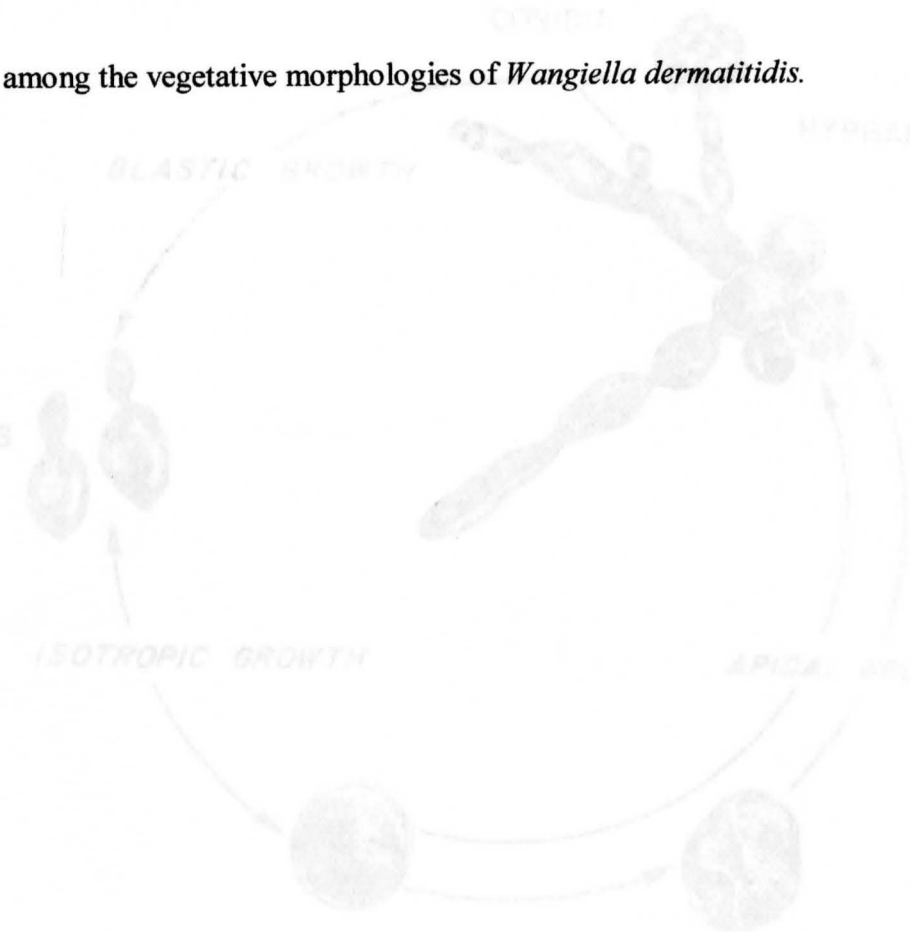
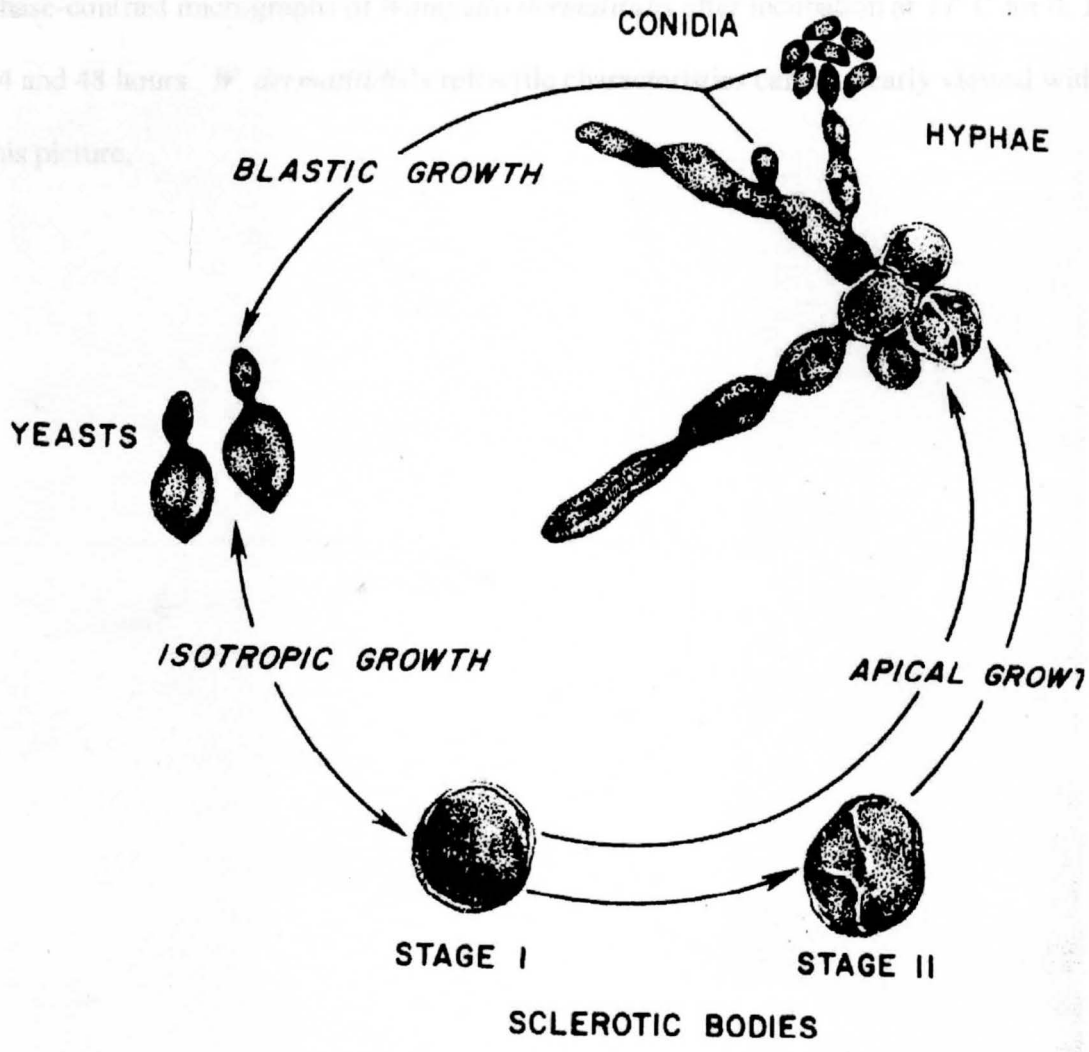


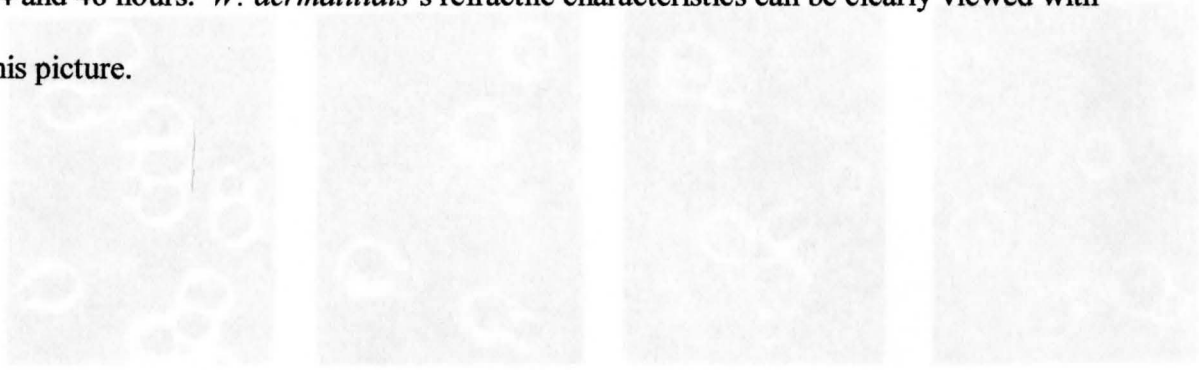
**Figure 1**




(Courtesy of P.J. Szaniszlo)

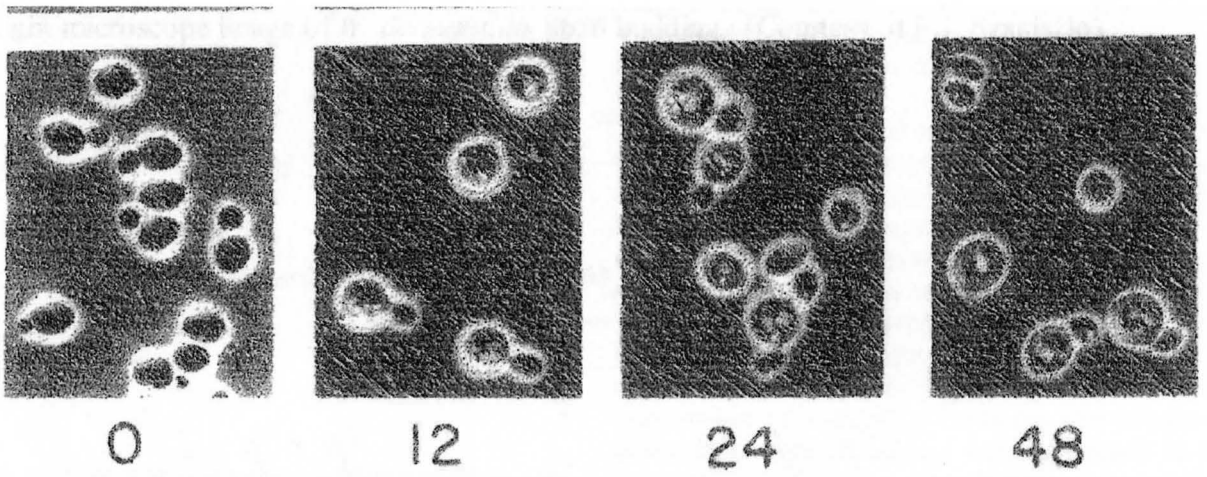
**Figure 2.**

Phase-contrast micrographs of *Wangiella dermatitidis* after incubation at 37° C for 0, 12, 24 and 48 hours. *W. dermatitidis*'s refractile characteristics can be clearly viewed with this picture.




(Courtesy of P.J. Szaniszlo)

**Figure 2**

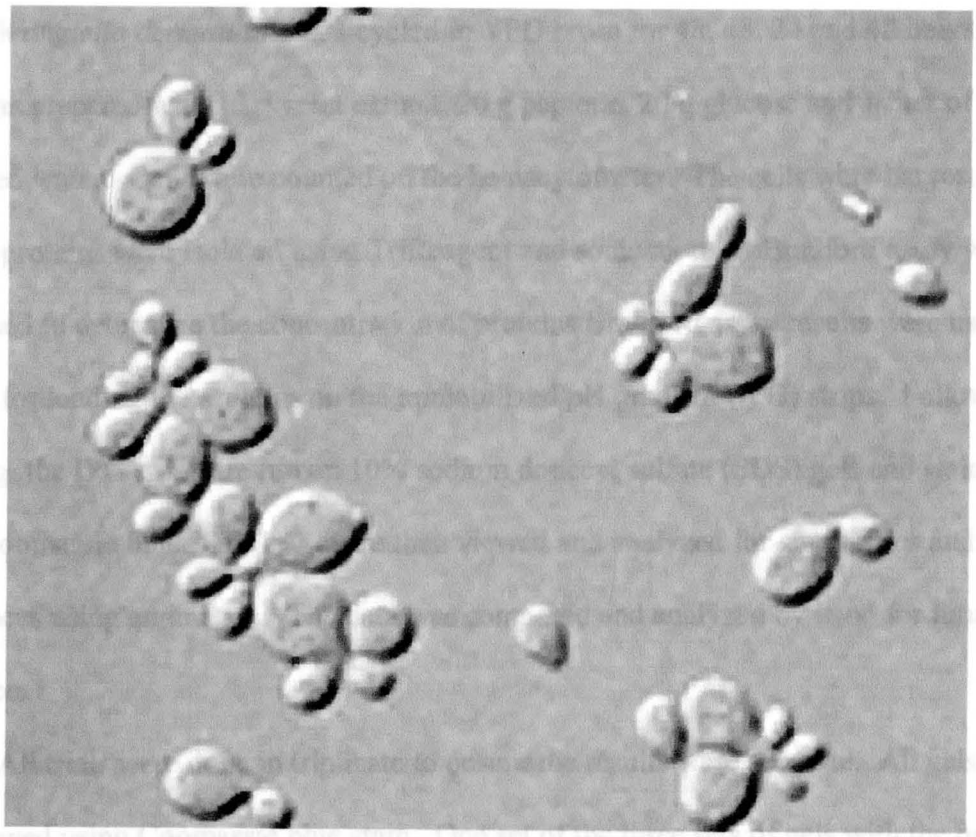



(Courtesy of P.J. Szanizslo)

**Figure 3**.

Light microscope image of *W. dermatitidis* 8656 budding. (Courtesy of P.J. Szaniszlo)

**Figure 3**



(Courtesy of P.J. Szaniszlo)

## Materials and Methods

General Overview of Methods

*Wangiella dermatitidis* was cycled in YPD broth for 48, 48, 24 and 48 hours. YPD was prepared with 10 g yeast extract, 20 g peptone, 20 g glucose and 1 liter of deionized water. Cells were counted on the hemacytometer. The cells were harvested and the proteins were isolated using TriReagent and sonication. A Bradford assay was performed to determine the concentration of proteins Bradford assay results were used as a guide for loading the proteins on the immobilized pH gradient (IPG) strips. Following focusing, the IPG strips are run on 10% sodium dodecyl sulfate (SDS) gels and stained using Coomassie blue. The gels were then viewed and analyzed for similarities and differences using an imager. The data was compared and analyzed by hand for future reference.

All trials were done in triplicate to ensure the results were accurate. All gels were first viewed using Coomassie blue stain. One set of the three sets of gels with the optimal amount of proteins observed, was also observed using Sypro Ruby staining. This sensitive stain proved to show many more proteins and would be an area of interest for future studies.

*Wangiella dermatitidis* Culture:

*Wangiella dermatitidis* strain 8656 was aseptically cultured from YPD slants (1% yeast extract, 2% peptone, 2% glucose, and 5% agar) and placed in a 250 ml flask with 25 ml of YPD broth (1%yeast extract, 2% peptone, and 2% glucose)(Szaniszlo et al, 1999). The culture was then incubated at 25°C for 48 hours. Cells were transferred from

broth to broth, using 0.5 ml of broth. The culture was transferred to a new 250 ml flask containing 25 ml of YPD. The process of cycling the cells for usage must occur at 48, 48, 24, and 48 hour intervals. At the end of the 24 hour period, the cells were counted in the hemacytometer and cells from the original flask were used to inoculate new flasks containing 25 ml of YPD broth at actual concentration of $1 \times 10^6$ cells/ml. The YPD broth was brought to the appropriate temperature before inoculation with the culture. These were then placed in their appropriate permissive (25°C) or restrictive (37°C) temperature range for the final 48 hours before harvesting.

Protein Isolation via TRI Reagent

TRI reagent (Molecular Research Center, Cat. No. TR 118) uses phenol and guanidine thiocyanate to inhibit RNase activity. After treatment with TRI reagent, chloroform was added to separate the solution into DNA, RNA and the lower organic protein layer. TRI reagent is one of the most reliable methods of macromolecule isolation from various organisms.

At the end of the cycles, 20 ml of the yeast culture was aseptically removed and placed into individual Sorvall tubes. The suspension was centrifuged for 10 minutes at 7500 rpm at 4°C. The supernatant was then poured off and the pellet was resuspended in 0.5 ml sterile TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.0). Next, 0.25 ml of the suspension was placed into Eppendorf tubes. The Eppendorf tube containing the suspension was spun at 7500 rpm for 10 minutes at 4° C. The supernatant was pipetted off and discarded. Each of the pellets received 0.5 ml of TRI Reagent and each tube was sonicated three times for 30 seconds then allowed to stand for 5 minutes at room

temperature. After 5 minutes, 0.1 ml of chloroform was added to each Eppendorf tube and shook vigorously by hand for 30 seconds. Each tube was allowed to stand for 15 minutes at room temperature, prior to centrifugation for 15 minutes at 12000 rpm at 4° C. Separation into layers occurred. The RNA remained in the aqueous phase, the DNA remained in the middle interphase and proteins remained in the lower organic phase. The lower protein layer was carefully pipetted into an empty Eppendorf tube. To each new tube, 0.75 ml of isopropanol was added and allowed to sit for 10 minutes while the proteins precipitated out of solution. The samples were centrifuged at 12,000 rpm for 10 minutes at 4°C and the supernatant was removed and discarded. The pellet was washed with 1 ml of guanidine hydrochloride/95% ethanol solution and allowed to sit for 20 minutes at room temperature. The samples were centrifuged for 5 minutes at 7500 at 4°C. This process of washing and centrifuging was repeated an additional 2 times. Subsequently, the supernatant was removed and 2 ml of 100% ethanol was added to the pellet, vortexed and allowed to sit for 20 minutes at room temperature. The sample was centrifuged for 5 minutes at 7500 rpm at 4°C. The pellet was air dried for 1 hour at room temperature. Finally, the protein pellet was dissolved with shaking and sonication in 1 ml of rehydration buffer. Rehydration buffer was prepared with 5.8 g/10 ml of 9.8 M urea, 400 mg/10 ml 1-4% Chaps, 23 mg/10 ml 15 mM DTT, 50 µl/10 ml of 0.2% Bio-lytes and 1 grain bromophenol blue per 10 ml.

Modified Bradford Assay

The Modified Bradford Assay was used to quantify the amount of protein contained in the solution (Bradford 1976). This assay utilizes the ability of Coomassie

Brilliant Blue dye to bind to the protein and an absorbance is measured at 595 nm. Following the recording of the absorbance, a standard curve of the absorbance versus the protein concentration or micrograms of protein was created. This linear relationship allowed for the determination of protein concentration in milligrams per ml.

The procedure used bovine serum albumin to create the standard curve. Sterile test tubes were labeled blank, 10, 15, 20, 25, 30, 40 and then with each of the sample names. To each test tube that did not contain sample the following was added: 10 μL of 2 dimensional electrophoresis (2DE) buffer, 10 μL 0.1 M HCl, 80 μL deionized water, 4 μL modified Bradford dye and corresponding amounts of bovine serum albumin. The 10 μL of 2DE buffer was added as a standard background for the absorbency and was composed of 8.4 M urea, 2.4 M thiourea, 5% CHAPS, 25 mM spermine base and 50 mM DTT (dithiothreitol). The tubes containing sample: 10 μL 0.1M HCl, 80 μL deionized water, 4 μL Modified Bradford dye, and 10 μL of sample were added. The samples were individually placed into sterile cuvette containers and analyzed via an absorbance of 595 nm to obtain the concentration of proteins present in 10 ml. The standard concentrations were placed in Excel and proved to be linear with an $R^2$ value of 0.95 or greater.

Analysis via Two-Dimensional Gel Electrophoresis

First, the proteins were resolved by isoelectric focusing. In the second step, the focused proteins were further resolved by molecular weight using electrophoresis on a polyacrylamide gel (Liebler and Yates 2002). These techniques allowed for a much clearer and more direct method of analysis.

Following the Bradford assay, calculations were done to ensure that 90 mg of protein were loaded onto IPG-ready strips with a wide pH range (3 to 10). The samples were diluted accordingly with rehydration buffer to achieve the desired 90 micrograms of protein per ml. After loading the samples and IPG strips into the tray, the samples were ready for active isoelectric focusing. The 7 cm or 17 cm IPG strips were rehydrated within the IEF. During the next 24 hours of run time, the strips were rehydrated within the IEF, run through a linear method with a 20°C focusing temperature and a limit of 50 μA per a gel resulting in separations of proteins based on their isoelectric pH. Following the 24 hour period, the strips were removed from the trays and were first placed face down in 2 ml of Equilibration buffer I with shaking for ten minutes. Equilibration buffer I was prepared with 3.6 g/10 ml of 6 M urea, 0.2 g/10 ml 2% of SDS, 2.5 ml/10 ml of 1.5 M Tris HCl with pH 8.8, 2 ml/10 ml of 20% glycerol and 200 mg/10 ml of 130 mM DTT. Next, the strips were placed in 2 ml of Equilibration buffer II with shaking for another ten minutes. Equilibration buffer II was prepared with 3.6 g/10 ml of 6 M urea, 0.2 g/10 ml 2% of SDS, 2.5 ml/10 ml of 1.5 M Tris HCl with pH 8.8, 2 ml/10 ml of 20% glycerol and 250 mg/10 ml of 135 mM iodoacetamide. Afterwards, the strips were laid over 10% SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) gels with an overlay of agarose. The 10% gels were composed of 48.0 μl water, 25.0 μl 40% acrylamide, 25.0 μl 1.5M Tris (pH 8.8), 1.0 μl 10% SDS, 1.0 μl 10% ammonium persulfate, and 1.0 μl TEMED. The gels were placed in TGS running buffer and run at a constant voltage of 16 milliamps per gel for the 7 cm strips and 10 milliamps for the large gels until the proteins migrated to the bottom of the gel, which was indicated by the overlay agarose. TGS buffer was prepared with 30.2 g Tris, 188 g glycine, 10 g SDS and

700 ml of water. Next, the gels were stained in Coomassie Blue stain for a period of 24 hours. After the 24 hours, the gels were destained in high destain for two hours and then shifted to low destain until the blue background color had been removed and the spots were clearly visible. The samples were then viewed using a Bio-Rad Gel ChemiDoc XRS imager. Pictures were taken using the imager and spots were analyzed by hand using Microsoft Paint to identify spots. Spots were identified on the basis of their level of expression.

SYPRO Ruby Staining

Gels containing significant proteins were duplicated, but stained with the more sensitive SYPRO Ruby stain. SYPRO Ruby stain detects minimally expressed proteins missed by the less sensitive Coomassie Blue staining techniques (Molecular Probes 2004). The gels were removed from the plates and placed in a fixing solution containing 300 ml of 30% ethanol and 50 ml of 5% acetic acid for a 1 hour period. The gels were removed from the fixing solution and put in Sypro Ruby overnight, which was followed by a 2 hour destaining period in a 10% ethanol, 6% acetic acid mixture. The gels were then fluoresced in the ChemiDoc XRS imager and pictures were taken for analysis. Analysis was done with PDQuest software.

## Results

Figure 4 shows the typical absorbency results of the BSA displayed in Microsoft Excel which were used to determine if the results of the Modified Bradford Assay were indeed linear with an $R^2$ of 0.95 or greater. Table 1 shows the protein concentration of the triplicate samples used in this study in accordance with $R^2$ value from the standard curve.

Figure 6 shows the raw unfiltered images of *W. dermatitidis* 8656 gels at 25°C and 37°C. The gels were run using the 3-10 pH gradient, 7 cm strips and a 10% SDS-PAGE gel. These were then stained in SYPRO Ruby stain. Vertical streaking was apparent on the gel. This can be edited out during analysis in PDQuest. Additionally, some speckling was apparent in the background, which can also be edited using the PDQuest software.

Figure 7 shows a comparison of proteins in *W. dermatitidis* 8656. These gels were stained with Coomassie blue stain. Gels were run on 3-10 pH gradient using a 10% SDS-PAGE gel. Blue indicates similar proteins between each of the gels, yellow indicates similar proteins on the gels and differences in the level of expression, and red indicates unique proteins. Between both of the gels, 9 unique proteins were found. There were also 21 similar spots matched, with 2 of the 21 having differences in the level of expression (indicated with yellow).

**Figure 4**

Results displayed in Microsoft Excel to determine if the results of the Modified Bradford Assay were indeed linear with an $R^2$ of 0.95 or greater. The results from the Assay are used to determine the micrograms per milliliter loaded for isoelectric focusing.

**Figure 4**

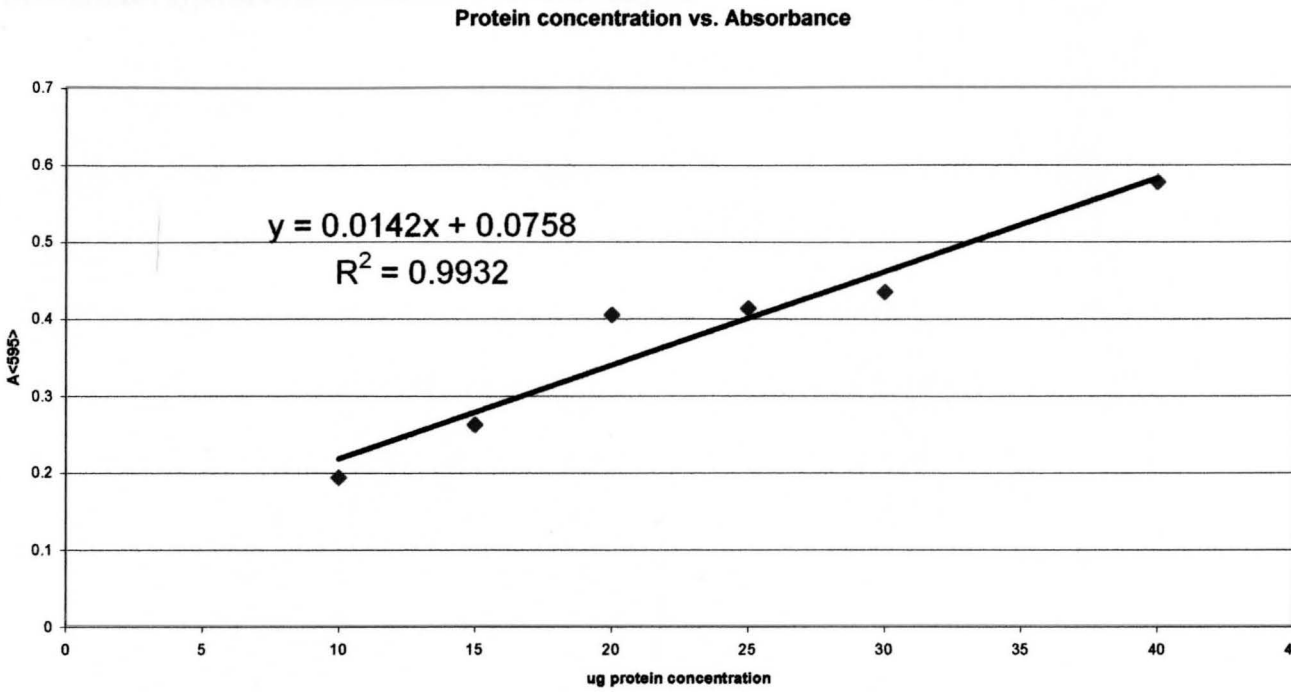

Protein concentration vs. Absorbance
y = 0.0142x + 0.0758
R2 = 0.9932
A<595>
ug protein concentration
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
5
10
15
20
25
30
35
40

**Table 1**

*W. dermatitidis* typical concentrations of protein samples.

Protein Concentrations of the Wild Type

| *W. dermatitidis* Sample | Absorbance at 595 nm | Concentration in μg/μl |
|---|---|---|
| Wild Type 8656 25 F1 | 0.13754 | 4.34 |
| Wild Type 8656 37 F1 | 0.18196 | 7.48 |
| Wild Type 8656 25 D2 | 0.14086 | 4.58 |
| Wild Type 8656 37 D2 | 0.12894 | 8.55 |
| Wild Type 8656 25 E2 | 0.13493 | 4.16 |
| Wild Type 8656 37 E2 | 0.19720 | 8.54 |

**Figure 5**

This image represents the method of loading the IPG strip, along with illustrating separation occurring as a result of the applied voltage.

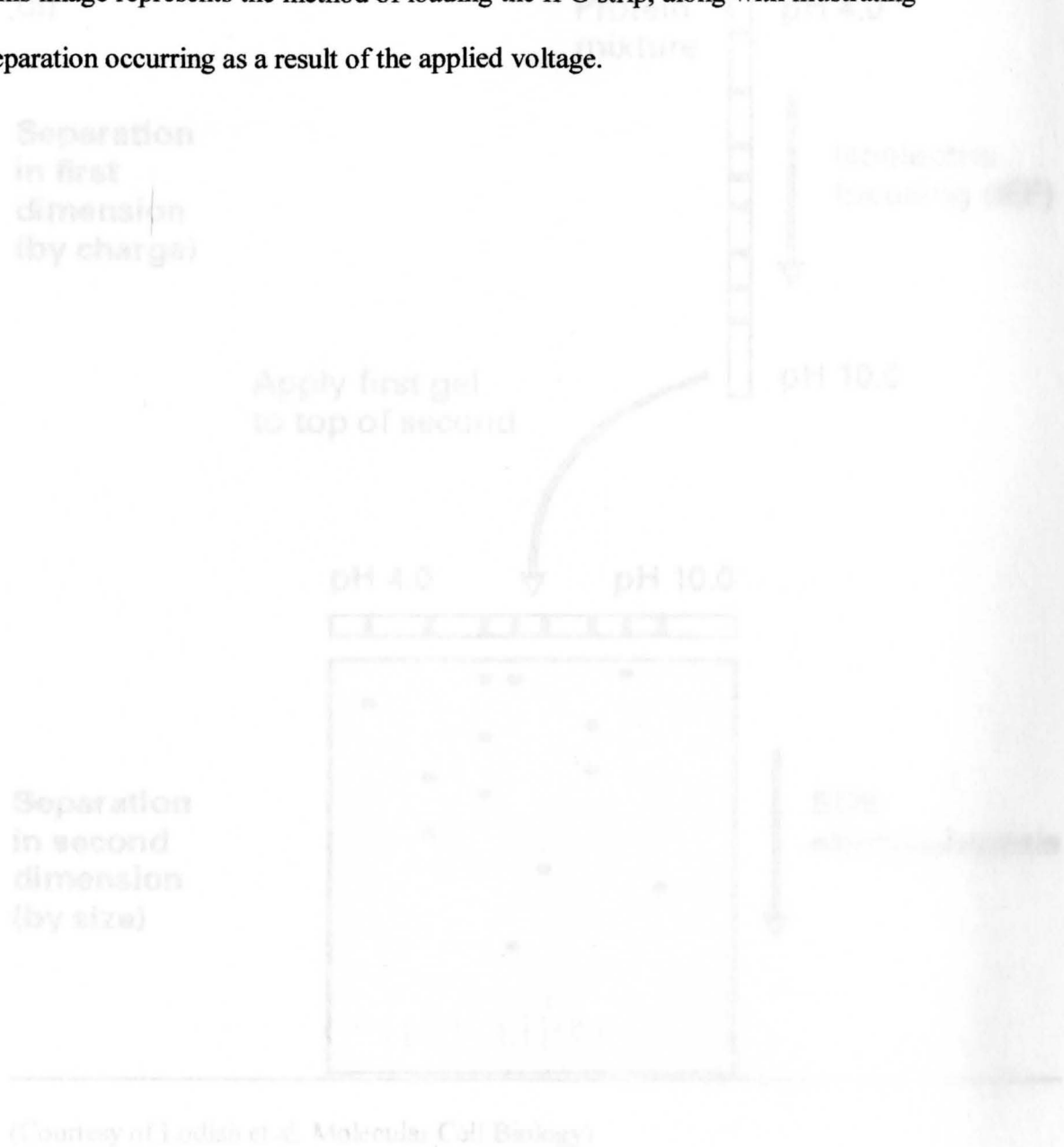
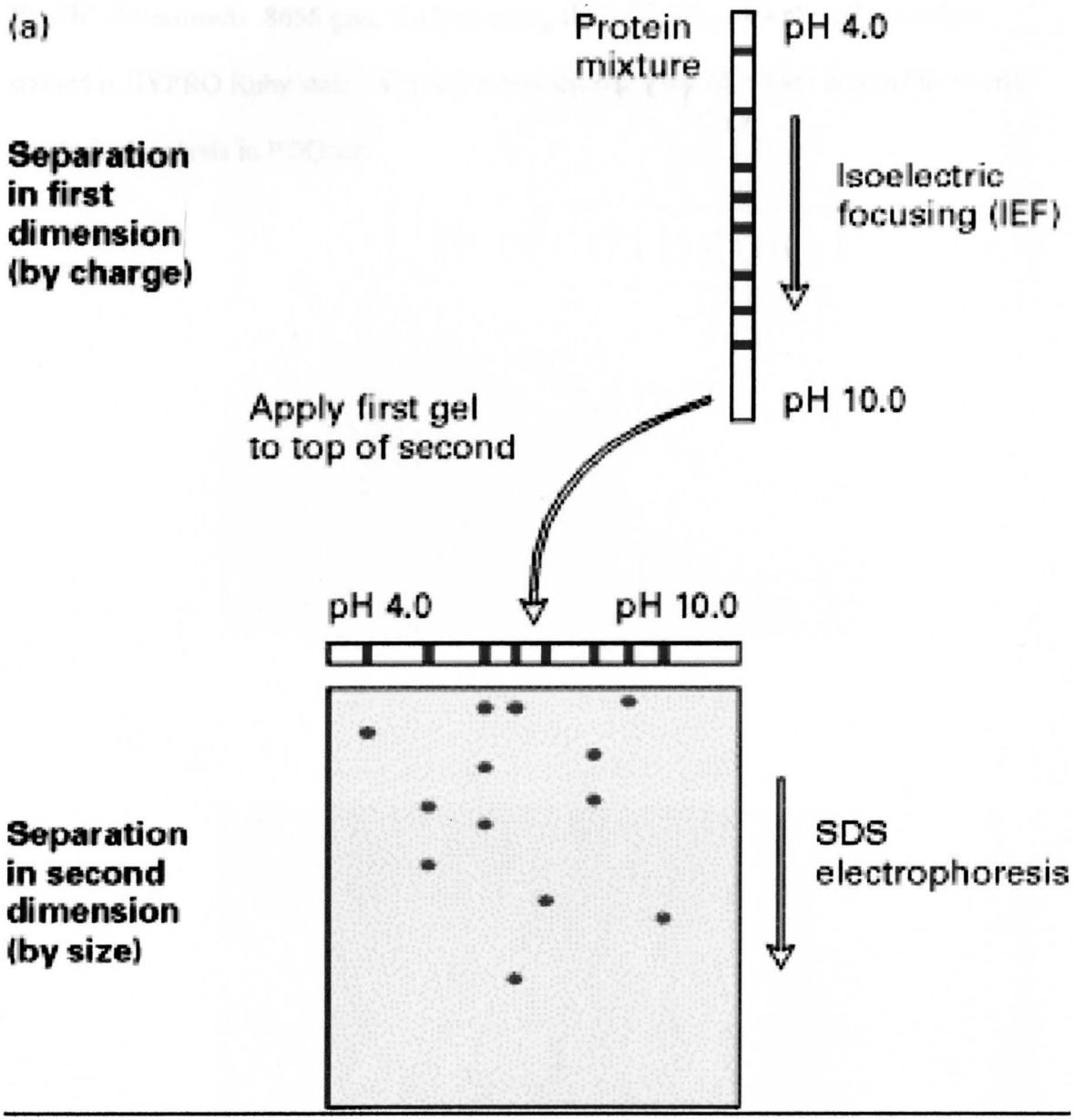


**Figure 5**

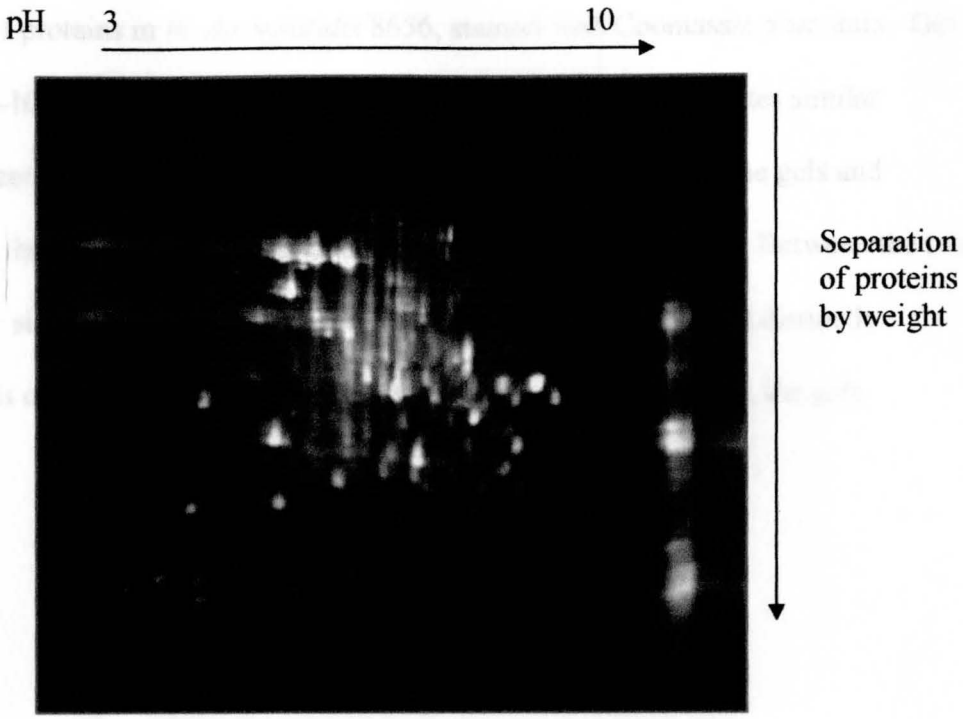



(Courtesy of Lodish et al. Molecular Cell Biology)

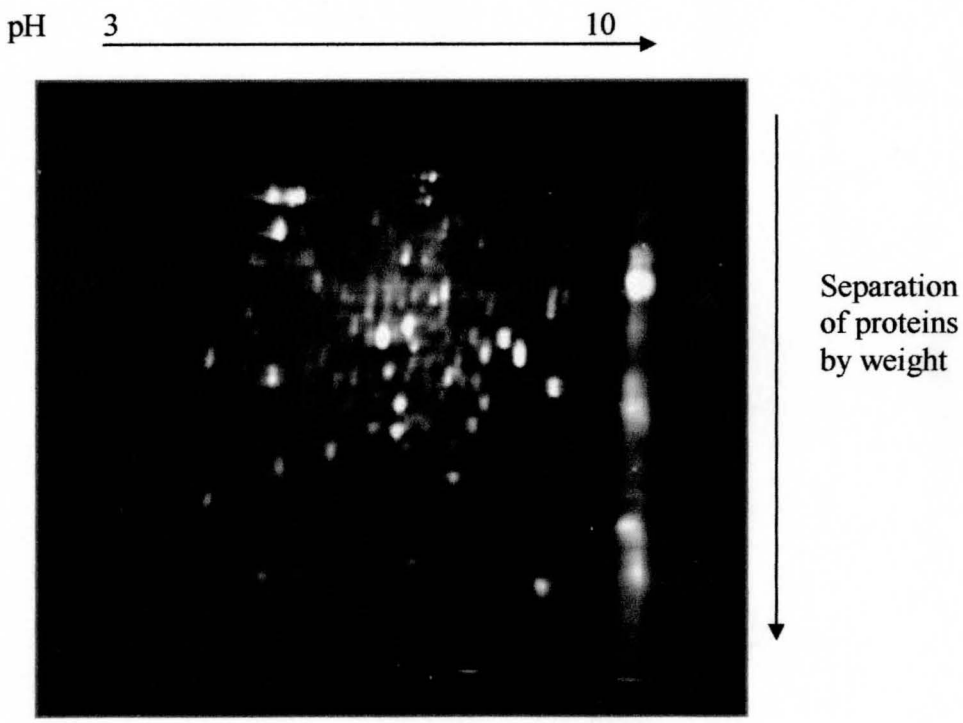
**Figure 6**

Raw *W. dermatitidis* 8656 gels. Gel ran using the 3-10 pH gradient on 7 cm strips, stained in SYPRO Ruby stain. Vertical streaking was present, which is possible to edit out during analysis in PDQuest.

**Figure 6**




Top: *W. dermatitidis* 8656 25° C, 7 cm gel

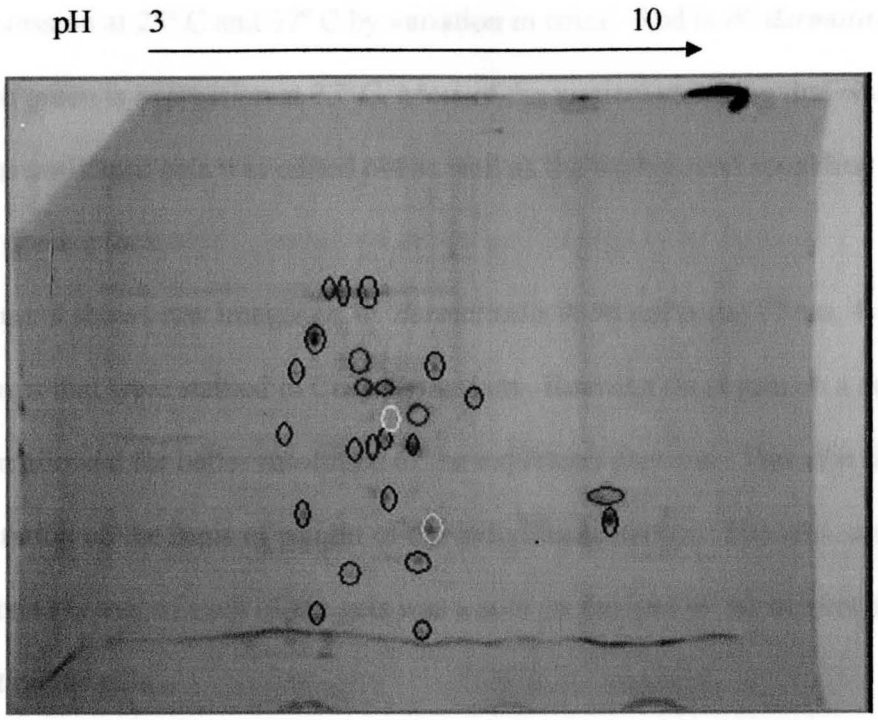



Bottom: *W. dermatitidis* 8656 37° C, 7 cm gel

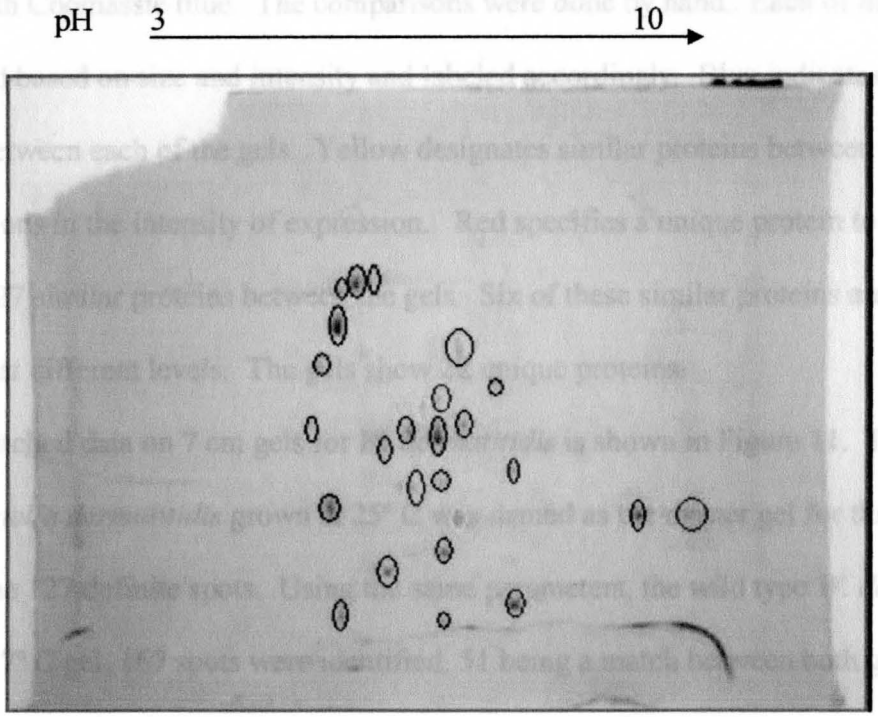
**Figure 7**

Comparison of proteins in *W. dermatitidis* 8656, stained with Coomassie blue stain. Gels were run on 3-10 pH gradient using a 10% SDS-PAGE gel. Blue indicates similar proteins between each of the gels, yellow indicates similar proteins on the gels and differences in the level of expression, and red indicates unique proteins. Between the two gels shown 21 similar spots were matched. Out of the 21 matched 2 had distinctly different levels of expression. Nine unique proteins were found between the gels.

**Figure 7**




Top: *W. dermatitidis* 8656 25° C, 7 cm gel




Bottom: *W. dermatitidis* 8656 37° C, 7 cm gel

Figure 8 was edited using PDQuest software. It illustrates the differences in the protein expression at 25° C and 37° C by variation in color. Red is *W. dermatitidis* 8656 at 25° C and green is expression at 37° C. Most of the vertical streaking that was seen in the previous unfiltered gels was edited out as well as the background speckling giving the gel a more precise look.

Figure 9 shows raw images of *W. dermatitidis* 8656 ran using 17 cm, 4-7 pH gradient strips that were stained in Coomassie stain. Running these gels on a narrower pH gradient allowed for better resolution of the expressed proteins. This also allowed for better separation on the basis of weight of the individual proteins. The spot seen in the lower left hand corner of each of the gels was a spot on the lens of the camera and was not present on the gels.

Figure 10 is a comparison matched proteins on the 17 cm gels from Figure 9, stained with Coomassie blue. The comparisons were done by hand. Each of the spots was judged based on size and intensity and labeled accordingly. Blue indicates similar proteins between each of the gels. Yellow designates similar proteins between the gels and variations in the intensity of expression. Red specifies a unique protein to the gel. There are 27 similar proteins between the gels. Six of these similar proteins are expressed at different levels. The gels show 22 unique proteins.

Matched data on 7 cm gels for *W. dermatitidis* is shown in Figure 11. The wild type *Wangiella dermatitidis* grown at 25° C was named as the master gel for this set, giving it the 127 definite spots. Using the same parameters, the wild type *W. dermatitidis* grown at 37° C gel, 167 spots were identified, 51 being a match between both gels. This leaves 116 unmatched unique proteins between the gels.

**Figure 8**

This image was created using the PDQuest software. It illustrates many of the differences in the protein expression at 25° C and 37° C. Red is *W. dermatitidis* 8656 at 25° C and green is expression at 37° C. Most vertical streaking that was seen in the previous gels has been edited out as well as most background speckling.

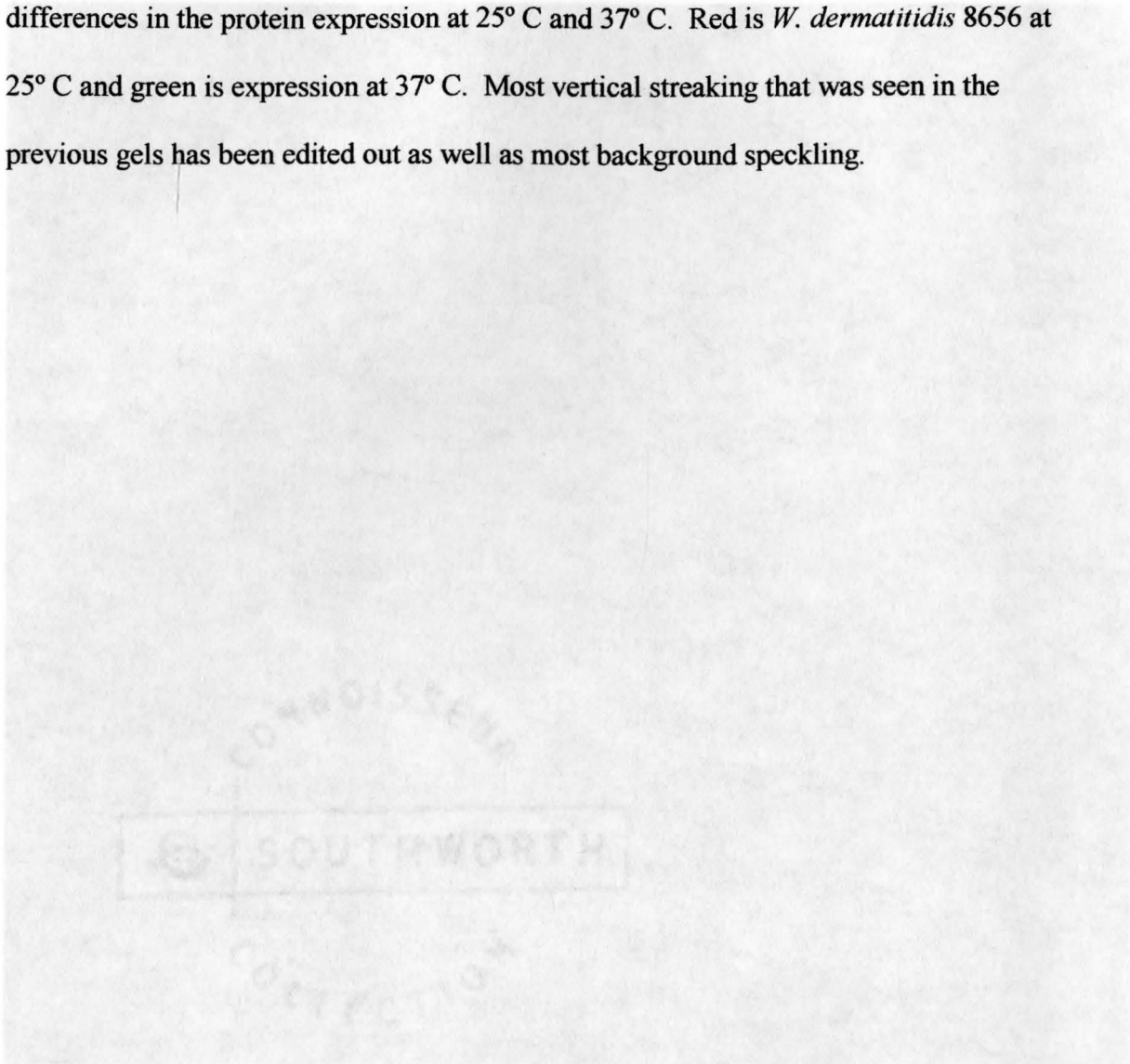
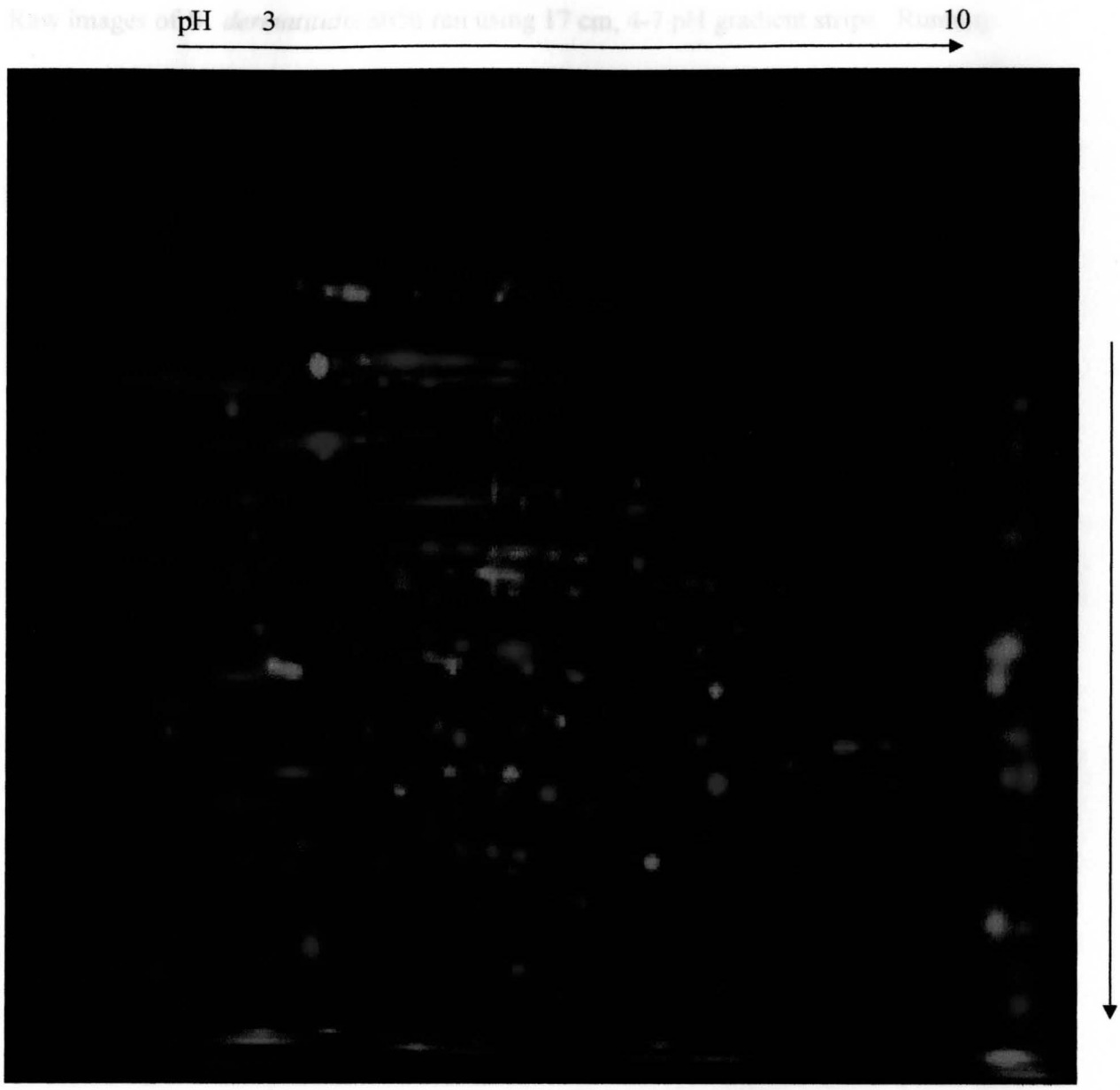


**Figure 8**




Over lay of *W. dermatitidis* 8656 at 25° C (red) and 37° C (green)

**Figure 9**

Raw images of *W. dermatitidis* 8656 ran using 17 cm, 4-7 pH gradient strips. Running the gels on a narrower pH gradient allowed for better resolution of the expressed proteins. This also allowed for better separation on the basis of weight of the individual proteins.

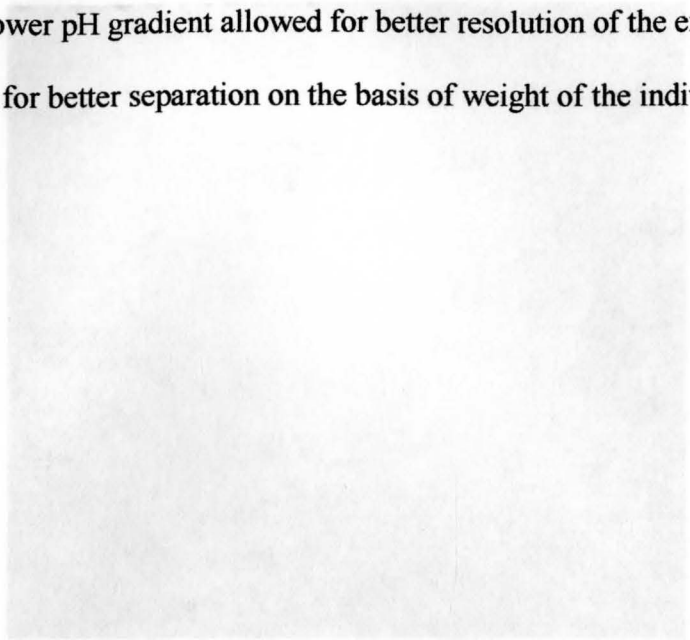
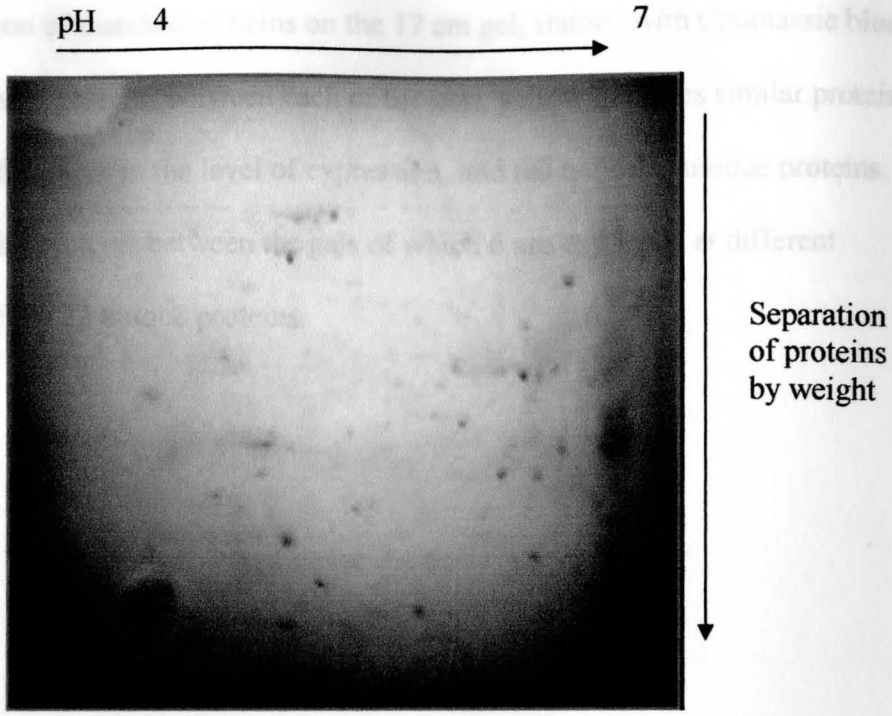
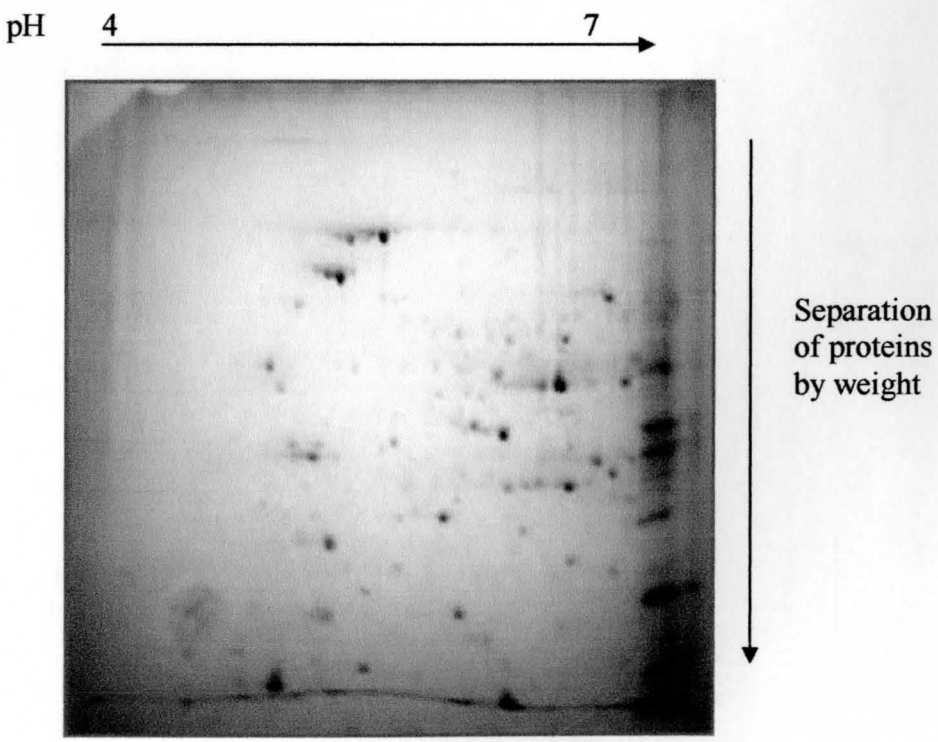


**Figure9**




Top: *W. dermatitidis* 8656 25° C, 17 cm gel

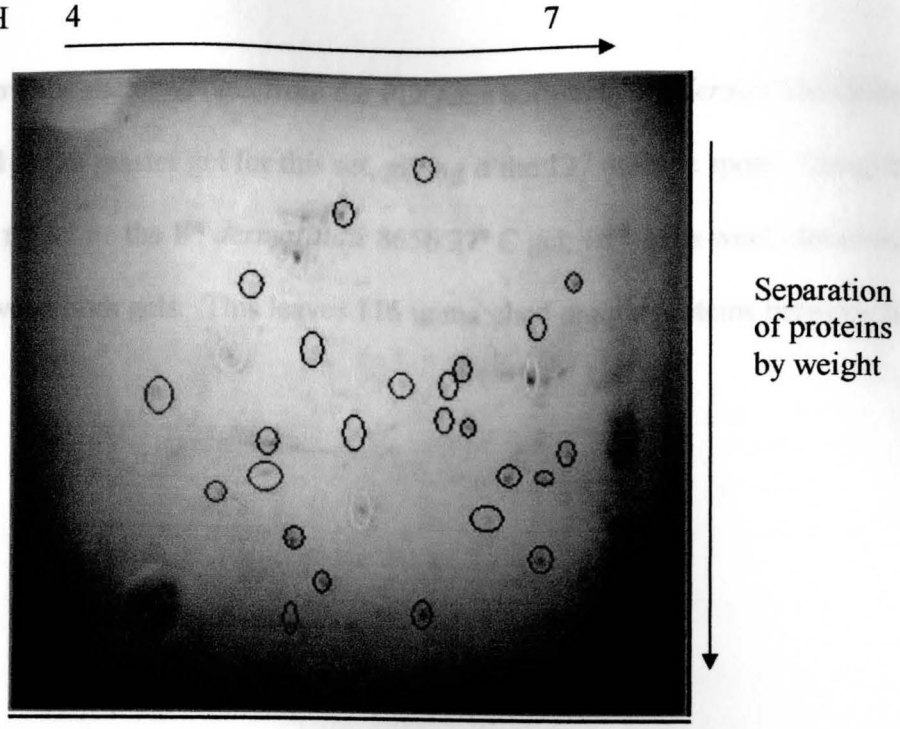



Bottom: *W. dermatitidis* 8656 37° C, 17 cm gel

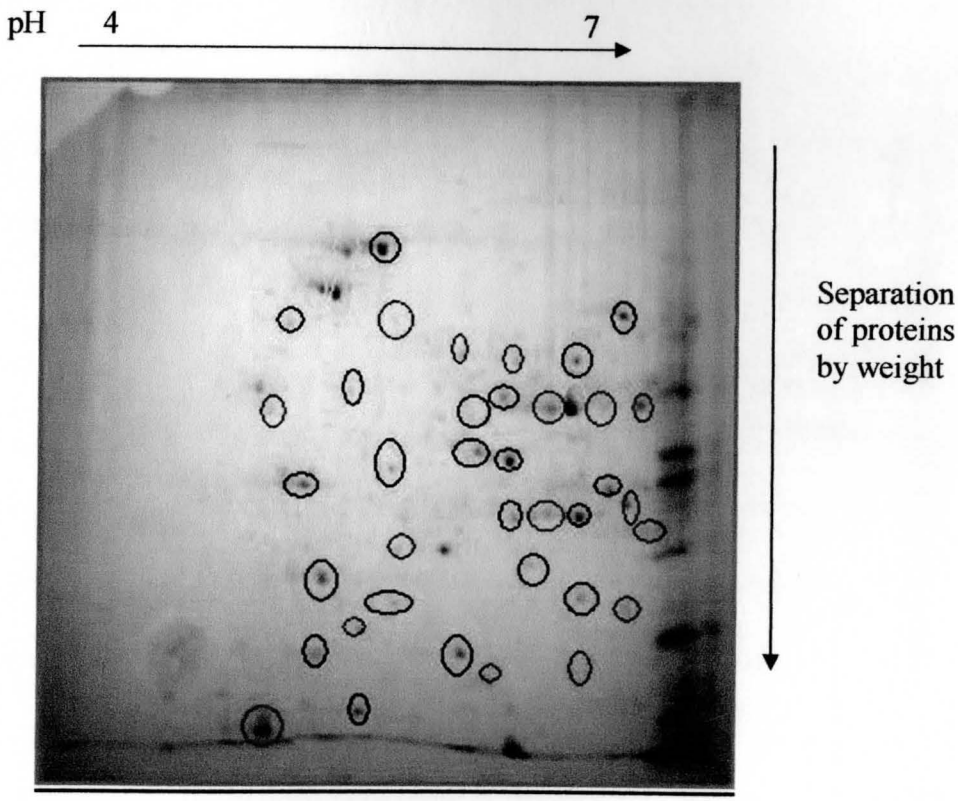
**Figure 10**

This is a comparison of matched proteins on the 17 cm gel, stained with Coomassie blue. Blue indicates similar proteins between each of the gels, yellow indicates similar proteins on the gels and differences in the level of expression, and red indicates unique proteins. There are 27 similar proteins between the gels of which 6 are expressed at different levels. The gels show 22 unique proteins.

**Figure 10**




Top: *W. dermatitidis* 8656 25° C, 17 cm gel

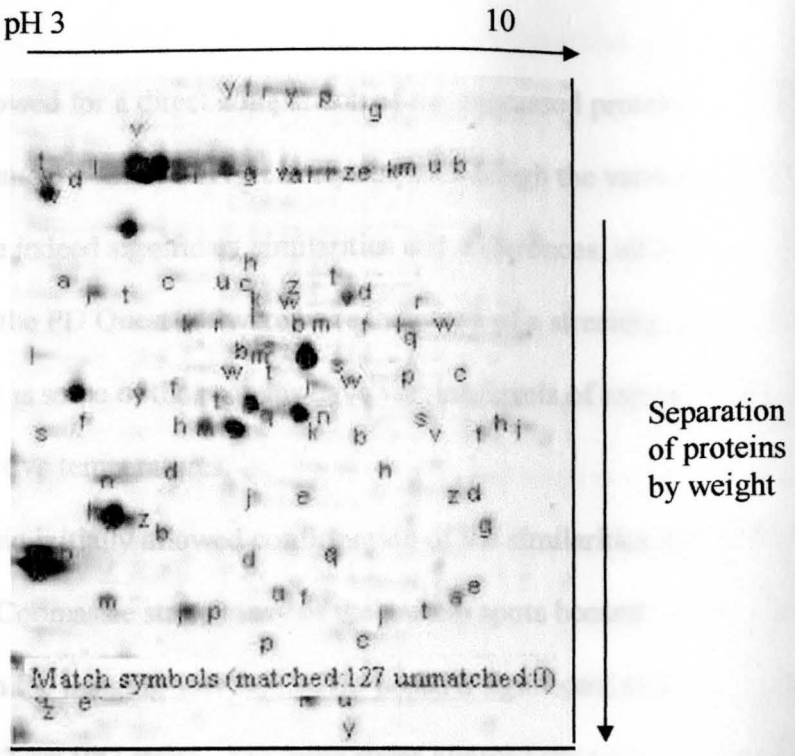



*W. dermatitidis* 8656 37° C, 17 cm gel

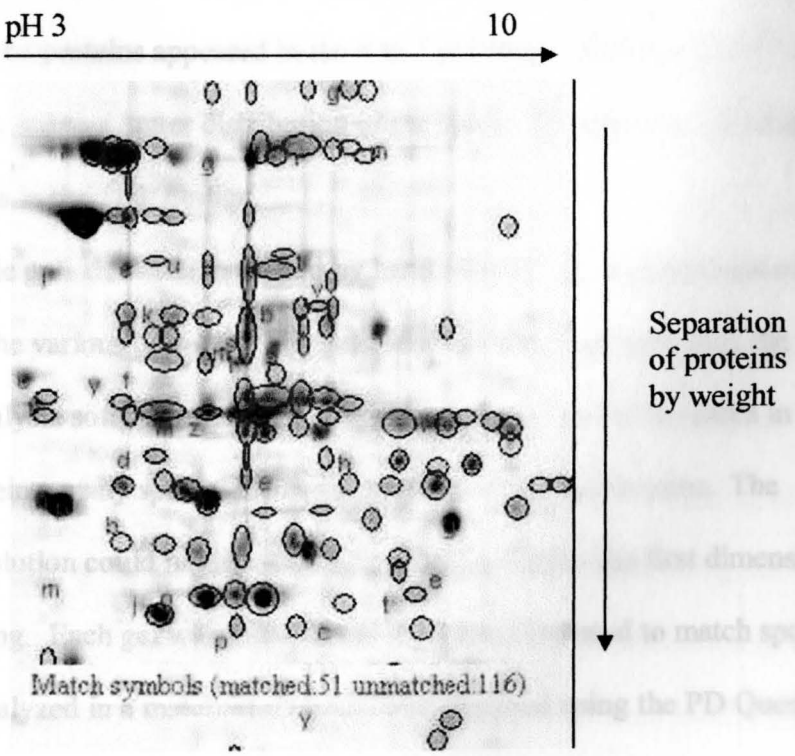
**Figure 11**

These figures show the matched data from the PDQuest software. *W. dermatitidis* 8656 25° C was named as the master gel for this set, giving it the 127 definite spots. Using the same parameters to define the *W. dermatitidis* 8656 37° C gel, 167 spots were identified, 51 matching between both gels. This leaves 116 unmatched unique proteins between the gels.

**Figure 11**




*W. dermatitidis* 8656 25° C, 7 cm gel




*W. dermatitidis* 8656 37° C, 7 cm gel

## Discussion

This study allowed for a direct comparison of the expressed proteins of *Wangiella dermatitidis* at 25° C and 37° C. It can be clearly shown through the various stains and software that there are indeed significant similarities and differences; although, some of the matches made by the PD Quest software were the result of a streaking on the gels. Even more interesting is some of the proteins have varying levels of expression in their permissive and restrictive temperatures.

Coomassie stain initially allowed confirmation of the similarities and differences. While destaining the Coomassie stain, many of the protein spots became so faint that they were hard to detect on the imaging software. This posed a significant problem and led to the use of the larger 17 cm IPG strips. The large strips allowed the proteins a larger area in which to separate and gave a better resolution. Also, observations were made during analysis that most of the proteins appeared in the 4 to 7 pH range. Running the 17 cm, 4-7 pH range IPG strips, led to a better distribution of the spots. The majority of the spots appeared on these gels in the 5 to 7 range.

The Coomassie gels that were analyzed by hand allowed for easy analysis of expression levels of the various proteins. The gels were initially matched using the PD Quest 2-D Image Analysis software but many variations arose. Due to variances in the resolution of the proteins, many spots were being matched or missed in error. The fluctuation in the resolution could be the result of errors focusing in the first dimension, speckling and streaking. Each gel was therefore first analyzed by hand to match spots. All gels were later analyzed in a matchset that was first analyzed using the PD Quest

software. Spots were then manually added or omitted. The proteins that significantly lacked expression are definitely an area of interest for future studies.

SYPRO staining proved to be an excellent choice for a more sensitive stain. The gels with the most numerous spots were rerun and stained with SYPRO. With the SYPRO stain at 25°C, 127 spots were identified. The 37°C sample had 167 identified spots. This is a significant increase in the number of detected spots in comparison to the first Coomassie stains that were analyzed. In the first Coomassie staining, 27 spots were found as opposed to the 127 found during Sypro staining. The limitation found in Sypro staining was that the sensitivity also produced a great amount of horizontal and vertical streaking. Again, the streaking caused the software to have difficulty in determining unique proteins as well as matched proteins. The PDQuest software and manual identification of the spots allowed the streaking to be removed. In future studies, trials will be run with various load sizes to see if this corrects the problem. Also care will be taken to ensure that the focusing in the first dimension is completed.

This research has lead to a vast number of future studies. Observing the better resolution of the gel images with use of the narrower pH range studies can be done in the future utilizing this fact. Along with this observation more time should be taken to stain the narrower pH range not only with the Coomassie blue stains, but also the more sensitive SYPRO Ruby stain and possibly silver staining. The SYPRO staining also offered the advantage of allowing for analysis on the PDQuest software using the UV light. This takes out for the concern of human inconsistencies in identification of the spots. It also allows for better analysis of the proteins and various other tools such as excision of the targeted protein. Once significant proteins have been identified within

either the 25° C or 37° C future studies may focus on excision of the protein for identification using mass spectroscopy. Also of interest is the comparison of the wild-type *Wangiella dermatitidis* to the multicellular mutant forms such as Mc3 and Hf1.

Finally, this study shows that there are indeed differences in protein prevalence in the restrictive and permissive temperatures of *Wangiella dermatitidis*. Also, the levels of expression were seen to differ in the varying temperatures.

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