

Corals coping with climate change: Identifying components of heat stress response in symbiotic cnidarians

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Abstract

Corals are the essential foundation species for coral reef ecosystems. Their reef-building capability is dependent upon a symbiotic relationship with an algal symbiont, in which the animal host exchanges waste products for photosynthate from the symbiont. When stressed, the symbiotic relationship breaks down and symbionts are expelled from the coral in a process called bleaching. Without symbiotic algae, corals are more susceptible to damage and death. Elevated sea surface temperature is the primary cause of coral bleaching, and it has been observed that bleaching events have been increasing as sea surface temperatures rise globally. We are interested in understanding the cellular events that lead to thermal stress-induced bleaching response. We exposed symbiotic sea anemones, *Aiptasia pallida*, to thermal stress (32°C for 24 hours) and used two-dimensional SDS-PAGE to compare protein profiles of anemones subjected to thermal stress compared to anemones in control conditions. The use of 2D protein gels required the development of new procedures to extract, purify, and quantify protein in anemone samples. Distinct differences in protein expression were found between heat stressed and control animals. The identity of these proteins will be predicted using MS to obtain peptide masses, and bioinformatic analysis to identify corresponding protein sequences from a database of *Aiptasia* genes.

Introduction

Background

Central to coral productivity and resilience is the symbiotic relationship between the coral animal and algae of the genus *Symbiodinium* that are housed within the animal's tissue. The algae, protected by the coral, fix carbon and function as a carbon source for the animal in exchange for waste products. The stability of this symbiosis, and therefore the welfare of coral reef ecosystems, is threatened by the global rise of sea surface temperature, because it causes the expulsion of the symbiotic algae in a process called bleaching (Figure 1).



Figure 1. Coral with bleached (white) and symbiotic (light brown) polyps.
<https://feww.wordpress.com/2008/05/26/>

Experimental System

The sea anemone *Aiptasia pallida* is useful as a system for modeling the coral-dinoflagellate symbiosis in the laboratory. Like corals, *A. pallida* establishes symbiosis with *Symbiodinium*. Lacking a calcareous skeleton, anemones are much easier to grow and maintain in the laboratory. Additionally, they will undergo bleaching when subjected to heat stress (Figure 2).



Figure 2. A symbiotic anemone (brown) and a bleached anemone.

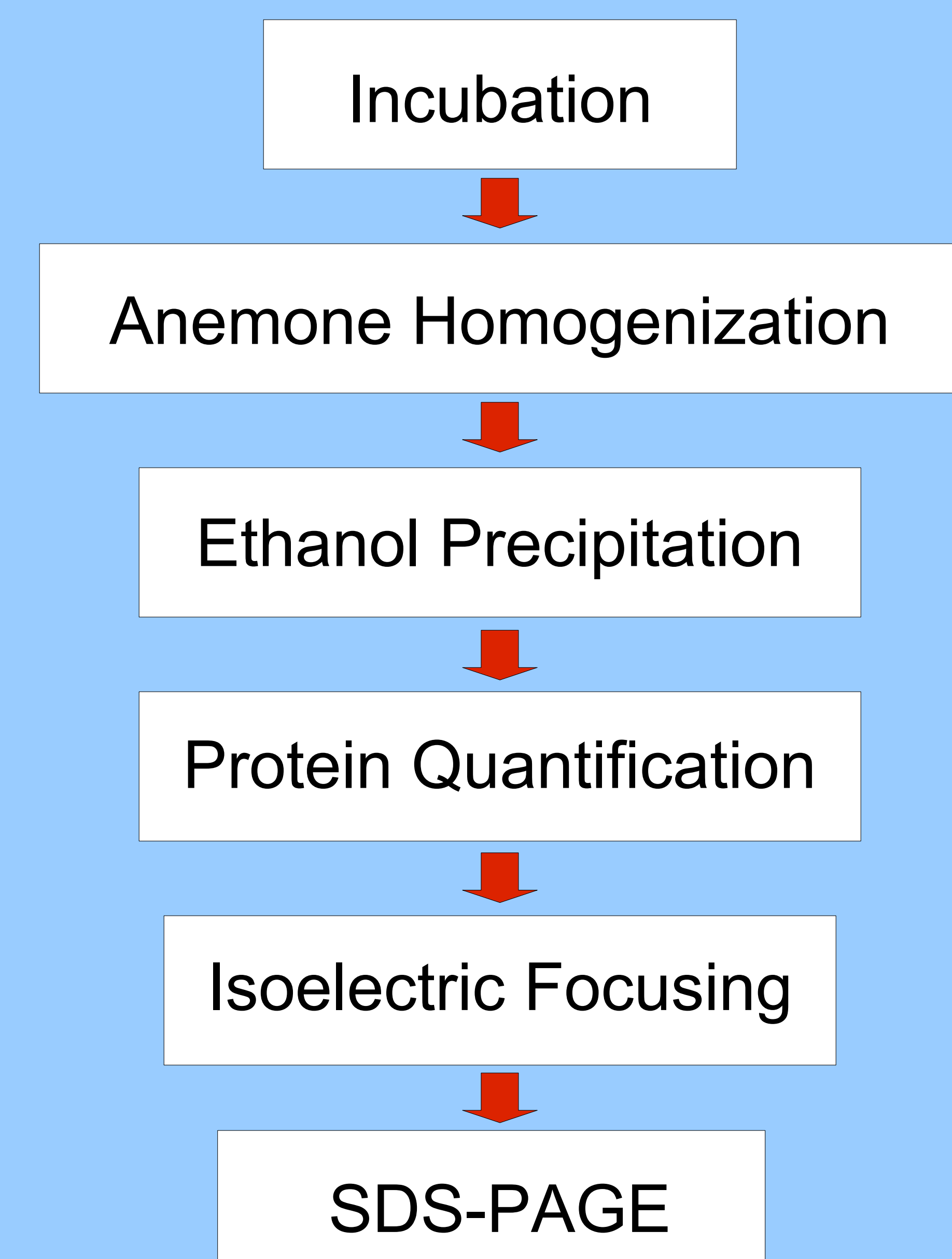
Hypothesis and Experimental Goals

The purpose of this study is to better understand cnidarians' response to heat stress at the cellular and molecular levels using a proteomic approach. Two dimensional gel electrophoresis (2D SDS-PAGE) was used to compare protein expression profiles of heat-stressed and control anemones and to separate proteins of interest so they can be identified using mass spectrometry (MS). We expect that among the up-regulated proteins will be enzymes that mediate oxidative and thermal stress damage such as catalase and heat-shock proteins, but we hope to identify proteins directly involved in the breakdown of the symbiotic relationship.

Materials and Methods

- Control anemones were grown at 27°C and heat-shock anemones were incubated at 32°C for 24 hours.
- Multiple anemones weighing 100-300 mg total were homogenized in a French press in the presence of SDS and a protease inhibitor.
- Samples were briefly boiled in SDS to solubilize proteins and centrifuged to remove impurities.
- Aliquots were mixed with 100% ethanol and frozen at -80°C for at least 2 hours and then centrifuged, producing an insoluble protein pellet.
- Pellets were dissolved in a lysis buffer containing 8M urea and protein concentration was determined by BCA assay.
- Proteins were separated according to isoelectric point (pI) over a range of pH 3- 10.
- Proteins were separated by molecular weight using SDS-PAGE on 10% polyacrylamide gels and visualized using Coomassie Brilliant Blue or fluorescent SYPRO Ruby stain.
- Spots were excised for MS and digested with the protease trypsin.

Experimental Scheme



Results and Discussion

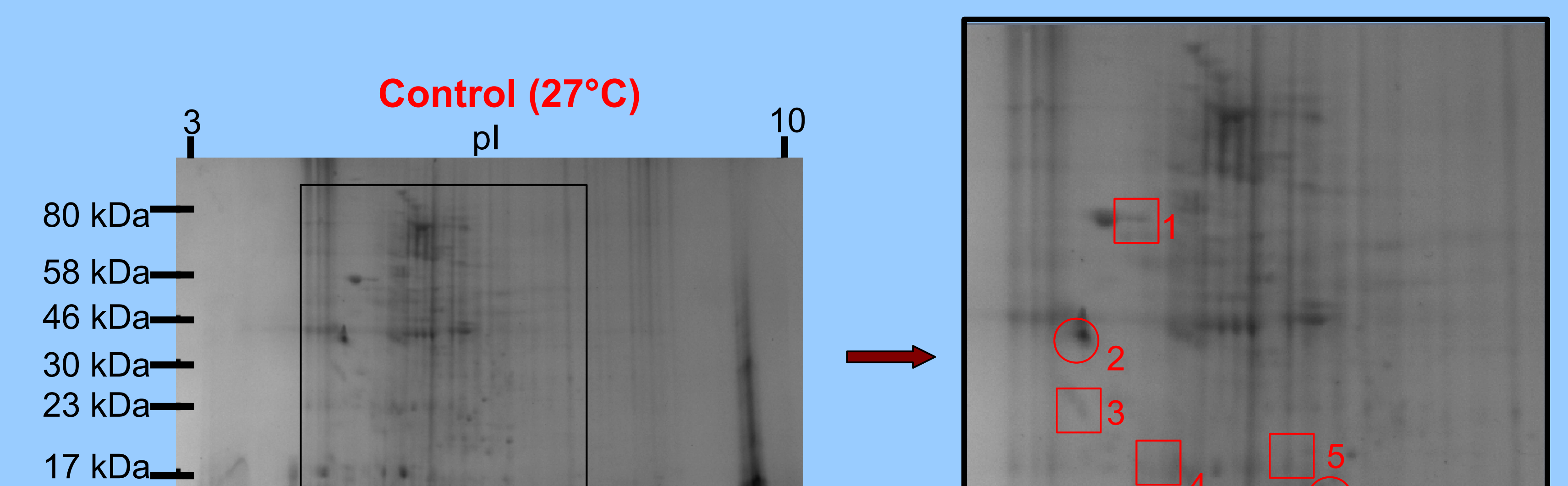


Figure 3. 2-D SDS-PAGE profile of room-temperature control anemones. 10% polyacrylamide gel stained with Coomassie Brilliant Blue.

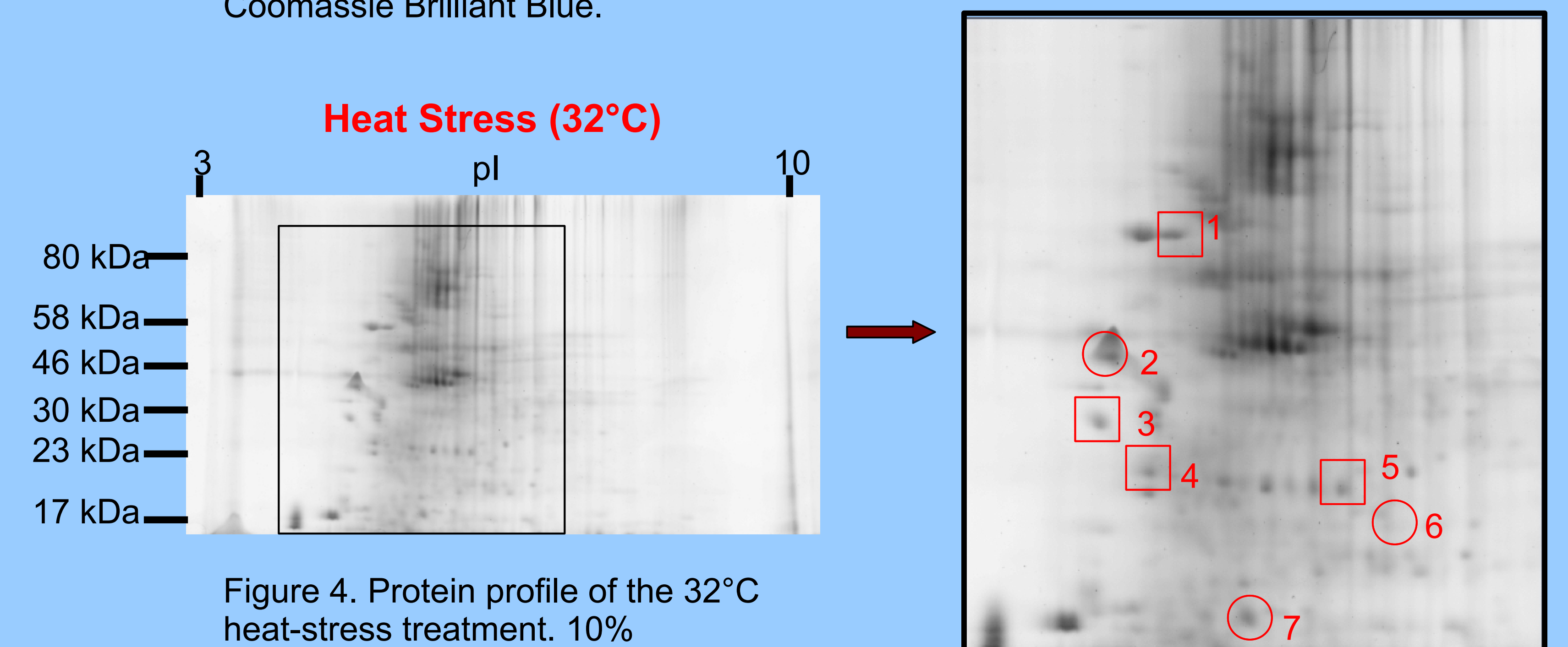


Figure 4. Protein profile of the 32°C heat-stress treatment. 10% polyacrylamide gel stained with SYPRO Ruby fluorescent stain.

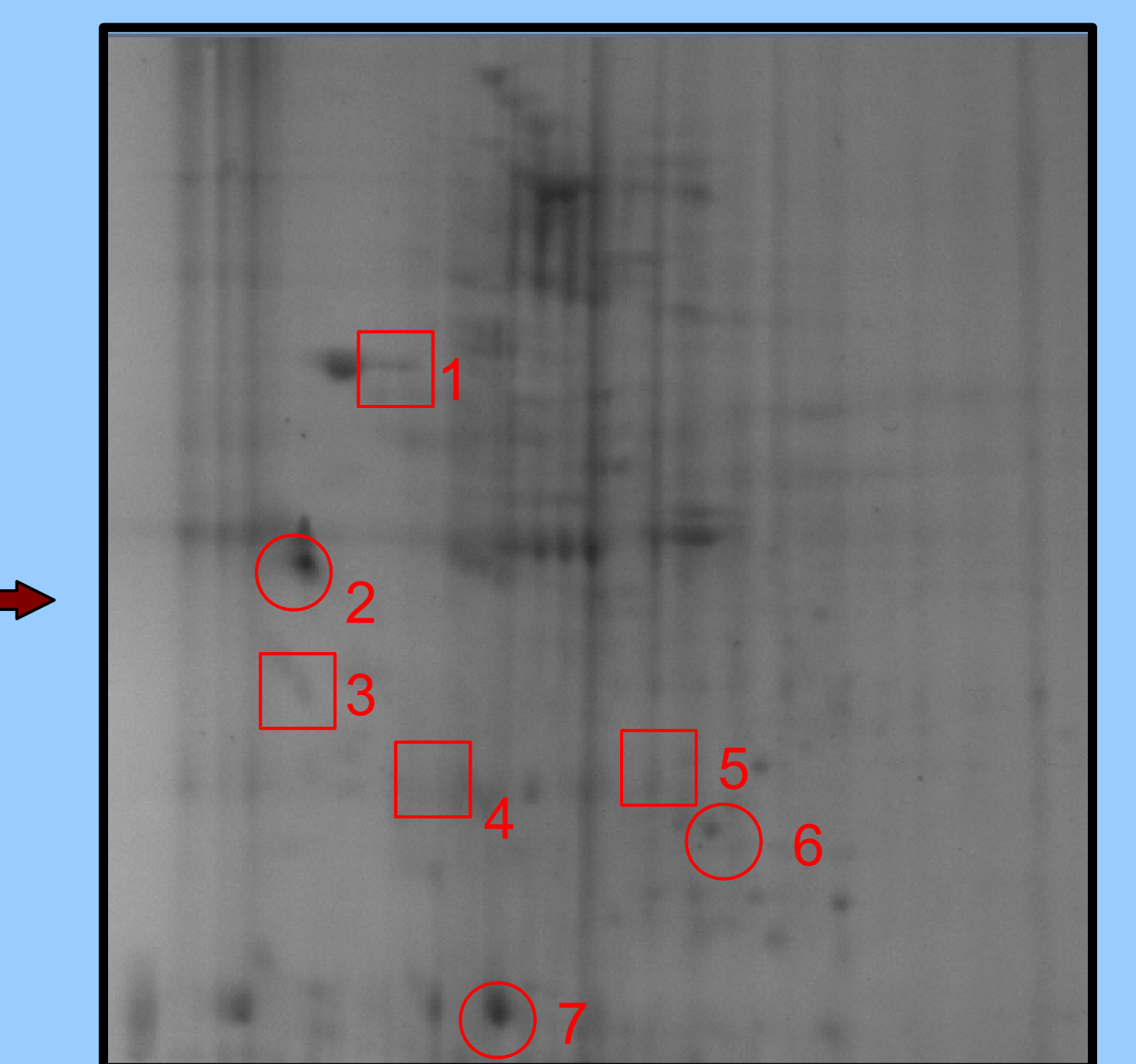


Figure 5. Identification of possible up-regulated (boxed) and down-regulated (circled) proteins

Comparison of protein gels from the two experimental conditions (Figures 3 and 4) revealed several spots that differ in intensity between the two, which may correspond to up- and down-regulated proteins. Spots 1 through 7 in Figure 5 were excised for identification by mass spectrometry.

Current and Future Work

Further research is needed to identify proteins involved in the breakdown of the cnidarian-algal symbiosis:

- Replication of 2D SDS-PAGE experiments will allow differential protein expression to be quantified by comparison of spot intensities.
- Excised protein spots will be sequenced using MS.
- Protein function will be predicted by comparison of sequencing data with known sequences in a database.

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