

## NIAC Phase I Final Report

Title of proposal: *Redesigning Living Organisms to Survive on Mars*

PIs: *Amy M. Grunden and Wendy F. Boss*

The hypothesis being tested is that one can revolutionize life forms by selectively expressing extremophile genes in plants, thereby enabling functional plant life in the most inhospitable environments, such as those on Mars. As proof of concept, the phase I goal was to produce functional extremophile proteins in plants. In addition, we proposed to engage an honors undergraduate class (ALS 398H) in a project to develop a preliminary design for virtual plants to survive on Mars.

**Results for Aim 1:** To test the concept that extremophilic archaeal genes could produce functional proteins in plants, we selected a gene encoding superoxide reductase (SOR) from the hyperthermophilic archaeon *Pyrococcus furiosus*. SOR is an enzyme that is functional at temperatures ranging from 4-100°C and provides protection from free radicals by reducing superoxide under anaerobic conditions. SOR is not found in plants and therefore, could provide a new mechanism for reducing stress-induced oxidative free radicals *in planta*. The plant system selected for the phase I trial was *Nicotianum tobacum* (NT1) suspension culture cells. NT1 cells were selected as a model system because they can be readily transformed and would in theory produce recombinant protein within the 6-month period.

The gene encoding *P. furiosus* SOR was amplified by a Polymerase Chain Reaction (PCR) using *pfu* DNA polymerase and the resulting PCR fragment was subcloned into the pENTR/SD/D-TOPO destination vectors. Using LR recombination, the SOR gene was then cloned into the plant expression vector pK7WGF2 (Functional Genomics Division of the Department of Plant Systems Biology, Gent, Belgium), which contains a green fluorescent protein (GFP) selectable marker. Recombinant plasmids were transformed into *Agrobacterium tumefaciens* EHA105 by the freeze-thaw method. *A. tumefaciens* containing either pK7WGF2-35S-GFP-SOR or vector control pK7WGF2-35S-GFP (control) were used to transform NT1 cells. The resulting cell suspension was plated onto NT1 agar culture medium containing kanamycin and timentin. The initial transformants were selected, transferred to 1 mL of liquid culture medium containing kanamycin and shaken at 120 rpm. The cells that grew on the selection medium were subcultured and maintained by subculturing weekly into 25 mL of NT1 medium containing kanamycin. For all subsequent studies cells were used 4 days after transfer.

As shown in Fig 1, the GFP-SOR fusion protein was present throughout the cytosol and in the nucleus of the NT1 cells and was not detected in the vacuole or nucleolus. The presence of the fusion protein was confirmed by preparing immunoblots of isolated protein from the transformed cells. As predicted, the 42-kDa fusion protein which was recognized by antibodies to GFP is detected only in the transgenic cell line. The cells were viable and grew at a rate similar to the wild type NT1 cells indicating that the recombinant SOR protein had no deleterious effect on cell growth under these conditions.

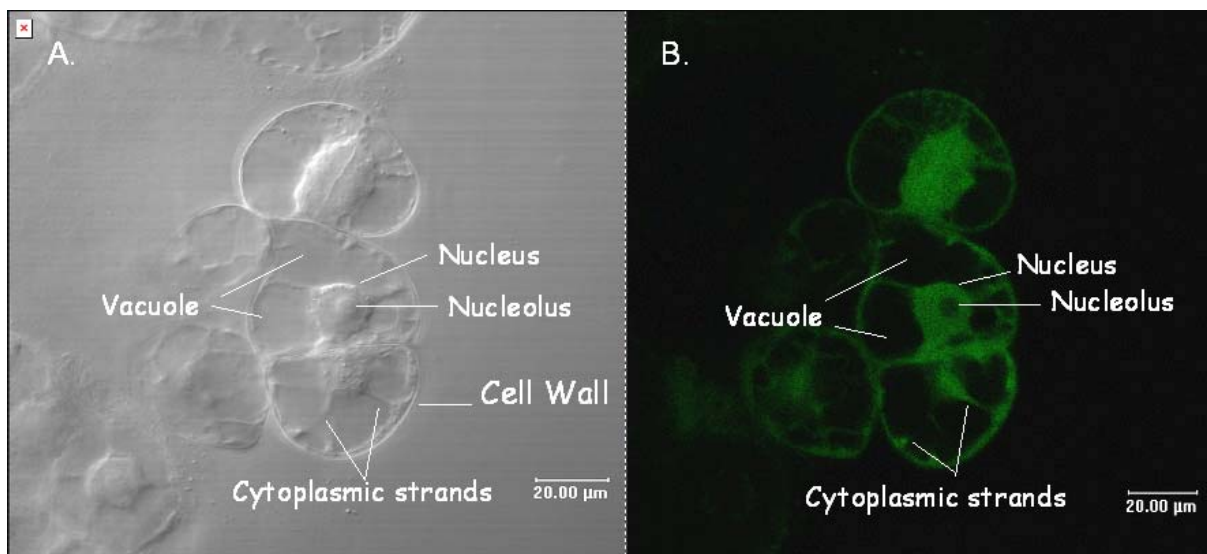


Fig. 1 A. Bright field image showing tobacco cells grown in suspension culture. B. Fluorescence image of the same cells showing GFP fluorescence in the cytoplasmic strands and the nucleus but not in the nucleolus or the vacuole.

Finally and most importantly, the transformed cells had SOR activity. Not only was there SOR activity, but the enzyme was heat stable, that is, the recombinant protein retained the properties of the native *P. furiosus* enzyme. The SOR specific activity data for the transgenic tobacco culture is presented in the Phase II proposal.

If SOR is functioning in vivo, it would generate hydrogen peroxide upon reduction of superoxide which in turn should stimulate the plant to produce catalase to detoxify it. Catalases are a class of redox enzymes that decompose hydrogen peroxide into water and oxygen. Initial studies of the SOR-expressing tobacco cells indicate that they have higher catalase activity than the wild type cells. These very preliminary data on the NT1 cells indicate that for optimal protection from reactive oxygen stress we need to express the next component in the *P. furiosus* SOR pathway, rubrerythrin reductase, to remove the peroxide without producing oxygen. The data also emphasize the need to do metabolic profiling of recombinant plants expressing these genes in order to identify compensatory pathways that might be expressed by the plant.

In summary, we completed our phase I goal which was to show that a gene from an extremophilic archaeon would produce a functional enzyme when expressed in plant cells and that the extremophilic enzyme would not prove toxic to the cells. To our knowledge, this is the first report describing the production of an archaeal protein in plants. We are currently writing up the results to submit for publication.

**Results for Aim 2:** Our second goal was to engage an honors class in a project to redesign plant life for Mars. The class (ALS 398H) was organized by AMG. Instruction in the course began on January 18<sup>th</sup>. Both PIs participated in the course to facilitate student learning. On April 26<sup>th</sup>, they gave an oral presentation describing their proposed greenhouse and plant material in the format of a mock NASA press conference (See

figure 2. Their Powerpoint presentation is attached as a separate pdf file). The students described how they would design their greenhouse, and how they would use microorganisms to help amend the soil for increased plant growth as well as to generate electricity to help power the greenhouse. They selected several species of plants they wanted to grow in their ecosystem, and identified several suites of genes to be engineered into these plants, which they proposed would increase tolerance to cold and drought. They also described how they proposed to set up experiments in the greenhouse to test different soils and to have biosensors to monitor when plants were stressed or needed water or oxygen. The students' final deliverable was a paper describing their concepts for redesigning plants both for life support facilities and as a means for terraforming Mars.



*Figure 2. The ALS 398 Honors students sharing their concepts for “Redesigning Plants to Survive on Mars” during their oral presentation on April 26, 2005*