

#05 Projects
Final Reports as of July 2008

#05-04 Commercialization of BmJ as a Broad Spectrum Microbial Disease Control Agent

Barry Jacobsen – Montana State University - Bozeman

\$104,400

Final report approved: September 2005

Summary:

A method to reliably produce BmJ spore preparations was developed and proven to meet EPA criteria standards. The formulation is in field trials in Michigan on cucurbits and carrots, in Montana on cucumbers, cantaloupe, greenhouse tomatoes, sugar beets and potatoes, and in Costa Rica on bananas, and will soon be in trials in Florida on tomatoes.

The assays developed to detect microbial contamination in BmJ spore preparations are rapid, economical, and meet EPA detection limits. Another assay necessary to meet EPA requirements for registration was developed to distinguish BmJ from other *Bacillus mycoides* isolates. The technique developed also provides the baseline for confirming the identity of the J isolate with subcultures of maintenance cultures.

A study showing no adverse effects from pulmonary exposure to BmJ spores removed the principal technical risk in obtaining EPA registration for BmJ. Extensive literature searches conducted by Montana Microbial Products (MMP) indicate *B. mycoides* is ubiquitous in the environment and is not reported as a human, animal or plant pathogen. This provides additional support for the safety of BmJ as a microbial fungicide.

The completed application for an EPA experimental use permit, which will allow for large-scale commercial trials throughout the United States, for the 2006 crop season, was submitted in August.

Delays in obtaining USDA/APHIS clearance to work on Black Sigatoga disease of banana at the MSU greenhouses were experienced. However, BmJ samples were shipped to a research pathologist with Dole Inc. in Costa Rica in fall of 2004. Initial trials were destroyed by storms, new trials initiated in April 2005, and results expected soon. Additional trials with the Costa Rican National Banana Corporation were started in late June and results expected in October/November 2005. The PI will provide results from both trials upon receipt.

Parallel projects funded by the USDA have demonstrated efficacy of BmJ against the cucurbit pathogens causing anthracnose, angular leaf spot and corynespora leaf spot.

Achievement of objectives:

This project has resulted in development of a method to reliably produce BmJ spore preparations that meet criteria for EPA Quality Assurance standards. An EPA Experimental Use Permit (EUP) application, which will allow for large-scale commercial trials throughout the United States, was submitted in August and it is anticipated these trials will occur in 2006.

Delays have occurred in getting clearance to work on Black Sigatoga disease of bananas at MSU, but BmJ samples have been sent to Dole Inc. in Costa Rica and to the Costa Rican National Banana Corporation. Results of both trials are expected soon and will be provided to MBRCT upon receipt.

Commercialization Plan:

The USDA awarded a Phase II SBIR grant for \$296,000 to Montana Microbial Products and MSU to support commercialization with expanded greenhouse and field trials, additional research on the BmJ mode of action in plants, and development of improved commercial formulations. Because of the positive aspects of the project, including continuing support from the USDA and MBRCT, MMP is moving forward with efforts to raise equity capital to support the cost of EPA registration and commercial introduction of BmJ.

#05-11 Imaging Studies to Test the Efficacy of Targeted Multi-Photon Photodynamic Therapy for the Noninvasive Treatment of Cancerous Tumors
Jean Starkey – Montana State University - Bozeman
\$160,263
Final report approved: April 2006

Summary:

Photodynamic therapy (PDT) is a promising non-invasive cancer treatment, which can be used after cancers have become resistant to chemotherapeutic agents. The overall goal of this project was to prove novel therapeutic triads work as efficient PDT sensitizers. The project also designed treatment protocols and verified that tumor cells could be treated at depth in tissue phantoms. The results were positive for all aspects of the project and the work has advanced to the point where Series A funding is expected. Two companies have expressed interest in partnering with Rasiris Inc. to develop “imagine driven PDT” instrumentation. Pacific Horizon Ventures, Seattle, has made a significant investment in developing the technology for breast cancer treatment and the underlying technology for developing two photon activatable sensitizers is patent protected with additional patents submitted or under review.

Performance Objective and Results of Studies:

- An undergraduate was hired and trained to work on the project.
- Results of studies following the uptake and clearance of compounds in the mice indicate there is a good window of time for PDT treatment when the tumor contains enough drugs, but no detectable drug is present in normal tissues.

- Bioluminescent human breast cancer cell lines were generated in the early stages of the project and used for the mouse studies during the rest of the project.
- Tumor response to PDT treatment proved to be easily monitored with the use of scanning for bioluminescence. A question raised during these studies, which will be addressed under another grant, is whether early PDT damage to the tumor vasculature can be monitored using the shut down of the bioluminescent signal.
- Experiments confirm that tumors are treatable at depth and that the laser is innocuous to normal tissues.
- The therapeutic triads effectively sensitized tumor cells in tissue culture and in mice for PDT treatment.
- The octreotate peptide effectively targeted the therapeutic triads to SST2 receptor positive tumors.
- Healing after PDT using the sensitizers was very good.
- Depth of possible treatment could not be assessed in an animal as small as a mouse. It is necessary to move quickly to studies using clinic dogs and cats.

Presentations & Manuscripts:

- 2005 & 2006 Photonics West meetings in San Jose, California
- 2005 American Association for Cancer Research, Anaheim California
- 2005 11th Congress of the European Society for Photobiology, Aix-les-Bains, France
- Two manuscripts regarding two-photon photodynamic therapy have been published.

Commercialization Plan:

MPA Technologies has licensed all technology currently being developed at MSU that relates to two-photon photodynamic therapy (PDT), which in turn re-licensed to Resaris, Inc., a joint venture company funded by Pacific Horizon Ventures (PHV). PHV is a Seattle venture capital firm specializing in the development of start-up biotechnology companies.

Product Description:

Two-photon PDT for the primary treatment of breast cancer and the development of an optical sentinel node biopsy

Production or manufacturing or strategic plan:

Current business model anticipates that MPA may manufacture and distribute PDT porphyrins during the pre-clinical stage of development. A strategic partnership with a major pharmaceutical company through the Rasiris licensing agreement will need to be developed as the project advances beyond mice. Co-development of a prototype imaging/two-photon PDT instrument platform with Resaris is in process and large animal studies may begin within the next 12 months.

MPAT will submit a NIH Phase 1 STTR in April to the new initiative on Image Related Therapeutics in collaboration with Dr. Starkey at MSU and the Veterinary Medicine School at Washington State University to test PDT instrumentation in large animal models.

Two-photon porphyrins are patent protected, a provisional patent for the imaging/treatment instrument platform will be submitted in February 2006 as part of the licensing agreement with Rasiris and additional PDT agents are under development and will be patented during the next two years.

Economic Impacts:

MPA Technologies will remain in Bozeman. Rasiris Inc. will remain in Bozeman, at least for the foreseeable future.

Increase in employment levels:

Immediate impact has been hiring of post-doctoral and student workers.

The project, if successful, will benefit cancer patients by providing cheaper, non-invasive options for cancer treatment. Benefit to states like Montana where provision of good health care to rural populations is an issue.

#05-14 New Fluorescent Dyes for Ultrasensitive Multiplex Detection in Proteomics, Biotechnology and Diagnostics

Edward Dratz – Montana State University - Bozeman

\$300,000

Final report approved: January 2007

Summary:

The objective of the project was to extend applied research to bring new, ultra sensitive proteomic detection dyes (Zdyes) to the point of commercialization. The Zdye Proteomics Platform will out-perform related products currently in use by a large margin in information content, reliability, and ease of use. This will result in increased cost-effectiveness for pharmaceutical development and for wide-spread research applications at academic and commercial centers.

The project intended to support the transition from an initial “proof of principle” Zdye to an initial set of Zdye products that can be marketed by a new Montana company, “Zdye Inc.”, for proteomics applications. The Zdyes have added value by synergy with the development of fluorescence measuring instrumentation by an existing Montana company, Resonon Inc..

The project successfully synthesized and alpha tested blue-excited, green-excited and red-excited Zdyes, and also prepared and demonstrated a green-excited Zdye that could be removed at the end of the separation. However, the chemical stability of the initial red-excited Zdye was not satisfying, which led us to delay in beta testing and initial sales, as a full three dye set is required for these activities. The new MBRCT #07-17 grant that started in July of 2006 is exploring additional chemical dye frameworks to prepare a more stable red-excited Zdye.

Commercialization:

1. Zdyes have been sent to Bio-Rad (a \$1B company) for testing in 1D gel detection experiments. The 1D gel detection requires dyes that can be made quite cheaply and the new red laser-excited Zdyes in preparation are promising for an initial market entry after additional testing.
2. The Molecular Probes Division of Invitrogen (a \$3B company) has committed to carrying out a detailed marketing study for Z dye 2D gel detection dye products in close cooperation with Z dye and at no cost to Z dye.
3. Perkin-Elmer remains keenly interested in Zdyes that can be cleaved from the proteins after separation by 1D and 2D LC methods, before mass spectral analysis.
4. Z dye is close to starting outside beta testing projects as new red laser-excited Zdyes are being developed and tested. An extremely capable set of laboratories experienced in applications of competing DIGE dyes have been identified as beta test sites.
5. It is expected that Zdyes will be available for initial sales in early summer 2007.
6. The Resonon 2D gel scanner development is proceeding well, with the support of an NSF STTR Phase II grant and Zdyes will start guiding the alpha and beta testing of this instrument as the engineering prototype comes on line.
7. Close working relationships have also been developed with Goldenhelix in Bozeman, seeking to elucidate protein patterns of health and disease and with Snidertech in Bozeman to more accurately and rapidly identify proteins and protein modifications from mass spectral data. Letters briefly outlining these relationships are attached to the final report.

Economic Impacts:

This project has been pivotal in the establishing and growth of Z dye LLC in Bozeman. Z dye is in its second year of a two year NIH STTR Phase I grant (\$200K/year) and has applied for a three year NIH STTR Phase II grant (\$400K/year). Z dye has also applied for a second NIH two year STTR for expansion of the technology into glycoprotein analysis. Two continuation in part patent applications have been filed by MSU on the Z dye technology and this IP has been exclusively licensed by Z dye LLC. The present project has helped Resonon to secure a two year NSF STTR Phase II grant for development of an engineering prototype of their Hyperspectral gel/microwell/microarray scanner that has been licensed by Z dye LLC for biological applications. Two major grants have been applied for by MSU based largely on Z dye technology, one to DoD to team with Veterinary Molecular Biology to better understand the mechanisms of immune stimulants using proteomic tools that is expected to be awarded early in 2007, and a second to NIH for development of systems biology at MSU (for \$10M over five years).

#05-18 Commercialization of Enhanced Plant Protein for Aquaculture Diets:

Production Scale Up and Trout Growth Studies

Clifford Bradley – Montana Microbial Products – Missoula

\$122,000

Final report approved: April 2007

Summary:

The project was part of a larger project using fungal culture technology to develop enhanced plant protein and plant oil to replace fishmeal and fish oil in aquaculture feeds.

Objectives of the project were:

- 1) Determine the best process equipment, systems and control options for commercial production of enhanced plant protein and oil using MMP scale solid culture technology.
- 2) Evaluate the effectiveness of feeding enhanced plant protein in supporting trout growth.

The project met the first objective by developing a fungal culture process for enhancing soy to meet nutritional criteria for replacing fishmeal in trout and salmon feed. The information gained was used to design, construct and test pilot scale production at a scale sufficient to support commercial feeding trials. The project has not met the second objective because feeds prepared with enhanced soy were not palatable to trout and much of the work focused on solving this issue. Three alternative approaches to improving palatability of enhanced soy are currently in trout feeding trials. The overall project is continuing with funding from the NSF and NOAA Phase II SBIR grants and MBRCT funding was crucial to obtaining these additional funds. Significant commercial potential exists for plant based protein and oils in aquaculture feeds. Replacing even a portion of fishmeal and fish oil in trout and salmon feeds would create a substantial market for enhanced soy and oil seeds.

Commercialization:**Product description**

Montana Microbial Products (MMP) plans to commercialize this technology by manufacturing and marketing nutritionally enhanced soybeans and or oil seeds to manufacturers of finished aquaculture feeds. The enhanced soy and oil seeds will replace fishmeal and fish oil now used as the protein and energy ingredients in feeds for farmed trout and salmon in initial target markets.

Target market including size

Aquaculture is a rapidly growing industry consuming 2.1 million metric tones of processed fishmeal per year, with consumption expected to reach 2.8 million metric tones per year by 2010. MMP will initially pursue markets to replace fishmeal in diets of farmed salmonids (trout and salmon), representing about 33% of the fishmeal used in aquaculture.

Marketing strategy

Market analysis, customer interest, technical support, and proximity all support initial market entry in the farmed trout industry in South Idaho. South Idaho accounts for more than 70% of US trout production and consumes about 15,000 tons of fishmeal worth about \$9 million per year, a market large enough to support initial manufacturing as a profitable business. Idaho sales will be followed by expansion in to other areas of the United States, then into farmed trout in Europe. Sales will be direct from MMP to feed manufacturers. Additional marketing efforts will include participation in industry conferences and publications.

Production or manufacturing plan

In concept, MMP will initially construct manufacturing capacity in production units of 20,000 metric tons per year.

Business risk assessment

Principal business risks include raising necessary financing, competing products, regulatory compliance, and protecting technology.

Estimate of sales revenues for five-year period into the future

Gross margins of 23 to 35% in the agricultural feed business are considered outstanding.

Economic Impacts:

The primary economic impact of this project was additional grant funding to support development of plant based aquaculture feeds. This grant provided the basis receipt of SBIR phase II funding from the NSF and for SBIR phase I and II funding from the NOAA. Total federal grants totaled about \$850,000 and supported expansion of Montana Microbial Products.

New businesses created or expanded

Receipt of additional SBIR funding from NSF and NOAA supported expansion of Montana Microbial Products.

Development of intellectual property

The project supported development of intellectual property around the process monitoring and control systems developed to maintain culture temperature, substrate moisture and to control spore formation and MMP is working to prepare patent applications for this technology. The technology has applications in Solid Substrate Culture of fungi for other process and products.

#05-20 A Commercial Software Package to Develop Fuel Maps from High-Resolution, Remotely Sensed Data

Robert Crabtree – Yellowstone Ecological Research Center - Bozeman

\$ 86,933

Final report approved: April 2006

Summary:

A commercial software package to develop fuel maps from high-resolution remotely sensed data has been created. Free distribution of the HIS/SAR algorithms and GUI is successfully bolstering YERC's scientific standing, and licensing or sales of LiDAR algorithm and GUI (ELF) to a commercial partner (RSI Incorporated, Boulder, CO) is being carefully explored. It would appear, at this time, work to fine tune the ELF will continue under a proposed SBIR Phase II.

Purpose:

Create a commercial software package to develop fuel maps from high-resolution, remotely sensed data.

Research Carried Out:

MBRCT funds helped complete algorithms and formalize and package the software steps necessary to create useable fuel maps based on the fusion of hyperspectral and synthetic aperture radar data.

Results and Findings:

- Algorithm development necessary to create the fuel-mapping product was completed.
- Decision Tree Analysis was selected for fusion algorithms.
- A custom Interactive Data Language-based workflow GUI was created instead of relying on the purchase and configuration of off-the-shelf software as originally planned.
- Custom IDL code was written to extract data to the desired geographic information system-compatible formats.
- A GUI was created for the commercialization of YERC LiDAR algorithms for determining height of both natural and man-made structures, and to determine the location of bare earth.

Commercial Potential of Research Results:

Three major modifications of product offering occurred and have resulted in free academic distribution of algorithms and GUI to bolster YERC's scientific standing and potential licensing or sales of LiDAR algorithm and GUI to a commercial partner (RSI).

Economic Impacts:

- Development of intellectual property has helped YERC improve its standing in the scientific and remote sensing communities.
- Employment levels have grown from ~four to ~12 between YERC and sister company, HyPerspectives, Inc.
- Funding has been received for other projects by diverse groups, such as NASA, NSF, National Park Service, the Air Force Research Lab, the Naval Research Lab, and NavAir.

#05-26 Nontoxic, Alkaline Sulfide Lixivants for Recovering Gold from Montana Orebodies

Corby G. Anderson – Montana Tech - Butte

\$125,350

Final report approved: January 2008

Summary:

The goal of this project was to expand fifty years of research activities initially funded by the Department of Energy through the Center for Advanced Separation Technologies (CAST). This project was undertaken to apply a basic understanding of alkaline sulfide leaching to industrial gold leaching. The overall goal of the project was to utilize the

alkaline sulfide leaching process for more effectively producing gold in Montana. This goal will continue to be pursued based on the goals achieved (listed below). A larger scale demonstration plant is needed to confirm for several entities interested in the technology. The specific goals of the project were successfully met.

Commercialization Plan:

The Final Report indicates commercialization continues as planned and that currently there are several interested global industrial parties who would like to see a larger and more modern demonstration plant capable of treating their gold ores. The focus is to design and build such a facility for testing and confirmation.

Current Economic Impacts:

- Graduate of two Master’s level engineers
- Assistance in the education of a PhD level engineer who joined the Department of Metallurgical and Materials Engineering at Montana Tech
- The next stage of the project, design and operation of a larger demonstration plant for confirmation should result in increased revenue streams and allow sustainability of three engineers at CAMP.
- Following confirmation of the larger scale demonstration plant and assuming arrangement of suitable terms with interested industrial entities, employment and revenue impacts will be significant for CAMP and the mining industry.

Note: The Final Report notes that CAMP is unique in that, as a University entity, it is entirely financially self-sufficient.

#05-29 Potential Health Applications of Sugar Beet Fiber
Howard Knapp - Billings Deaconess Clinic Research Center - Billings
\$407,429
Final report approved: July 2008

Summary:

The purpose of the grant was to explore the potential health aspects of sugar beet pulp, specifically to study the sugar beet fiber on blood cholesterol. The primary end point would determine whether sugar beet fiber causes reduction in blood cholesterol in free living subjects who are attempting to follow an American Heart Association diet.

Conclusions of the study include:

- Adequate amounts of sugar beet fiber will lower blood cholesterol.
- The effect is the same as that seen for oat fiber.
- Significant reductions were seen when medication was added on top of continued sugar beet ingestion.
- Initial mechanistic studies suggest that sugar beet fiber is lowering cholesterol mainly by binding bile acids and preventing their recycling.

- Production and utilization of a high purity sugar beet fiber would result in a value-added component for sugar beet growers.

Commercialization:

Clinical results conclude that sugar beet fiber will lower blood cholesterol. Discussions with animal feed producers in the Billings assure indicate sugar beet pulp is being used for animal feed and is not going to waste. Thus, it is likely that producing a high purity sugar beet fiber would result in a value-added component which would benefit sugar beet growers. Preliminary discussions have been held with the Montana business Incubator at MSU-Billings and the Big Sky Economic Development Authority to explore development of the commercial potential for sugar beet fiber production in Montana. This will necessitate an economic assessment of the practicality and cost of obtaining patent licenses

Economic Impacts:

Although the mechanistic aspects of the sugar beet fiber lowering of LDL cholesterol are still not defined, the study has validated the fact that it does lower cholesterol. This fact seems to warrant exploration of the economic aspects of developing a high purity sugar beet fiber as a nutraceutical that may prove useful in combination with statins for treatment of the early stage of cardiovascular risk. This would bring added value to the sugar beet industry. The next step will be to advance the health benefit of sugar beet fiber by applying for a patent and acquire investment capital to expand the effort.

#05-30 Development of a Nerve Stimulation System for Clinical Use in Regional Anesthesia and Pain Management

Philip C. Cory – Nervonix, Inc. - Bozeman

\$115,000

Final report approved: June 2006

Summary:

This project represents a collaborative effort between Nervonix and Strategix Vision to design, prototype, and produce a needle system under patent protection of intellectual property by Nervonix. In addition, Nervonix has a one-year agreement (from June 2005 to June 2006) with Stryker Instruments for evaluation of the system. A complete description of the component electrical parameters was a reasonable accomplishment and the mathematical description will be pursued beyond this grant. Prototype designs developed by Strategix Vision were used by Stryker to produce working prototypes of the hub output control nerve stimulating needles and these prototypes have been well received in the anesthesiology community.

Commercialization Plan:

Stryker has licensed the intellectual property related to the nerve stimulation system and commercialization is expected to proceed through Stryker Instruments as part of a regional anesthesia system.

Product description

The system may be implemented in stages and would comprise three components: (1) nerve imaging capability; (2) nerve stimulating capability; (3) and, single shot and catheter placement capability where a catheter could be placed through the stimulating needle for continuous regional blocks.

Target market including size

Potential market size is estimated to be between 11 million and 12.5 million peripheral nerve blocks performed annually in the United States. In order to capture a significant portion of this market, the system would need to be seen as economically advantageous while improving clinician efficiency and patient comfort.

Marketing strategy

Marketing strategy individualized for a particular institution or type of institution would necessitate economic analyses. Reports of this kind of analysis indicate up to \$3,000 saved for joint replacement surgery performed with regional anesthesia as opposed to general anesthesia; \$400 savings for each patient bypassing Phase 1 post-anesthesia care, and \$400 savings for each patient discharged to home on the day of surgery.

Business risk assessment

Ultrasound is the only technology currently being marketed as an aid to regional anesthesia. Although images can be impressive, they can be difficult to interpret, are expensive and requires a steep learning curve for use.

Economic Impacts:

Patents applied for or granted

Intellectual property has been patented and is currently under license to Stryker Instruments during the evaluation period.

Development of intellectual property

This work has contributed to additional proprietary knowledge and has become part of the Nervonix intellectual property portfolio.

#05-31 Enhancement of Applied Research in Biomedicine

Richard Bridges, University of Montana, Missoula

\$375,000

Final report approved: September 2007

Summary:

This report marks the conclusion of the 3-year performance period (i.e., 2 years of funding and a 1 year no-cost extension) for the third award (#05-31) that the MBRCT made to the NIH Center for Biomedical Research Excellence (COBRE) in Structural and Functional Neuroscience (CSFN). The project continues the success of three previous MBRCT awards in furthering the objectives of increasing a critical mass of researchers and building the infrastructure necessary to increase the competitiveness of biomedical

research efforts in Montana. To that end, and as part of a five-year collaborative effort through the NIH-funded COBRE award (\$9,411,644 for 2005-2010), the project has increased jobs through the acquisition of federal research grants and contracts, enhanced collaborations with private sector companies, developed and protected intellectual property that has the potential for commercialization by existing or new companies, and been the vehicle for start up of four spin-off biotech companies, Big Sky Biotechnologies, TranSynaptic, ATERIS, and GT Pharma Inc. In addition to the COBRE award, over \$11 million in the past year alone has been received from federal research contracts. This is a significant economic impact because it represents new money coming in to Montana. Continuations of the project have been awarded MBRCT funding, most recently in June 2007. These funding decisions by the Board attest to the accomplishments achieved and the potential for significant economic impact to Montana in the future.

Commercialization plan:

The purpose of this project is to develop infrastructure in the area of applied biomedical research and, therefore, does not fall easily into the typical commercialization model. It is appropriate that the Final Report does not address this section specifically.

Economic Impacts:

The narrative of the Final Report indicates the project, as a continuation of several previous projects, is positively impacting economic development in Montana within the area of applied biomedical research.

New businesses created or expanded

Four spin-off companies – Big Sky Biotechnologies, TranSynaptic, ATERIS, and GT Pharma

Increase in employment levels

Project has contributed to the salaries of more than 70 employees, including 30 students.

Sales generated

Project has brought in more than \$13 million in federal research contract dollars in the past year.

Patents applied for or granted

Five provisional patents were filed during the award period.

Development of intellectual property

Intellectual property has been the foundation for the establishment of four new Biotech companies, Big Sky Biotechnologies, TranSynaptic, ATERIS, and GT Pharma Inc.