Idaho Incubation Fund Program

Final Report Form

Proposal No. **IF11-016**
Name: **Kenneth Cain**
Name of Institution: **University of Idaho**
Project Title: **COMMERCIALIZING SPECIFIC PROBIOTIC BACTERIAL STRAINS AS DIRECT FED MICROBIALS (DFMs) TO IMPROVE FISH HEALTH AND REDUCE DISEASE RELATED MORTALITY AT AQUACULTURE FACILITIES**

Information to be reported in your final report is as follows:

1. Provide a summary of overall project accomplishments to include goals/milestones met, any barriers encountered, and how the barriers were overcome:

   This project set out to address a number of objectives aimed at better defining specific probiotics bacteria and their characteristics in relation to potential commercialization. The following objectives were established:

   1. Optimize growth and production parameters for C6-6 and C6-8.
      a. Scale up current conditions to 10L or more using a laboratory scale fermentor.
      b. Optimize aeration, temperature, and inoculation conditions to maximize growth.
   2. Determine appropriate storage conditions to minimize loss in bacterial viability while keeping costs at a minimum.
      a. Concentrate bacteria in Fish Oil (currently used when mixing into feed) and test viability following storage (-20°C).
      b. Test lyophilization (freeze drying) of bacteria as an alternative.
   3. Test equal mixtures of C6-6 and C6-8 under laboratory and field conditions their ability to reduce CWD.
   4. Test feeding frequency and dose requirements to achieve desired benefits.
   5. Develop a complete business plan.

   We have achieved goals outlined in the initial proposal and over the eighteen months, two graduate students (David Burbank and Tyson Fehringer) were supported on this project. Both student pursued an MS degree and David finished in June of 2011 and Tyson is expected to graduate in December of 2012.

   Nearly all objectives have been addressed at this time. Growth and production parameters have been tested and it was demonstrated that production scale up

would not be an impediment to commercialization. In addition, storage conditions have been better defined and it has been shown that lyophilizing the bacteria is possible, but initial efforts did show a reduction in viability. We are continuing to test other lyophilization conditions to minimize impacts on bacterial viability.

We have completed initial testing of C6-6 and C6-8 alone and in combination. A field trial was set up at a Utah Division of Wildlife Hatchery; however, test fish in that hatchery never experienced a CWD outbreak making interpretation of results inconclusive. A recent laboratory trial was completed, but mortality differences were not apparent due to our disease challenge being too harsh, resulting in high mortality in all groups. This is not surprising as previous work has shown that the benefits of these probiotics can be masked if a heavy infection and severe disease outbreak occurs. We are repeating the above laboratory experiment and will be challenging fish by a more natural waterborne exposure method, which we have recently been successful in developing for CWD in rainbow trout (unpublished data).

Testing of feeding frequency and dose requirements was not completed but is planned once we establish which probiotics bacterial strain (alone or in combination) provides the greatest benefit.

A business plan was developed by students in the College of Business and Economics. This plan provides a foundation for development of a start-up company or direction and marketing trends useful for potential company partnerships.

It should be noted that in addition to the objectives developed for this project (listed above), we completed additional experiments and tests viewed as important for further development and commercialization of these aquaculture probiotics. One experiment that was completed was aimed at identifying the effects of probiotics on the immune response in trout. When exploring regulatory processes and requirements for such products, it was suggested that we investigate the potential mechanisms associated with probiotics benefits and determine if this were related to enhanced immunity in fish following feeding. If immune enhancement could be demonstrated it could allow these to be classified as biologics and regulatory approval then sought through USDA. However, our trials did not show obvious immune enhancement and this will likely require us to register our product as a feed additive through FDA.

Finally, in the process of laboratory testing on growth and storage conditions, we decided to see if one of our strains (C6-6) was capable of inhibiting another related fish pathogen. What we found is that it was able to inhibit the growth of *Flavobacerium columnare in vitro*, which is important since this is another significant pathogen of salmonids and a major pathogen for warmwater aquaculture species such as catfish and tilapia. This finding suggests that

application of these probiotics may be much broader than originally anticipated and could expand the market substantially.

2. Describe the current state of the technology and related product/service:

   Our early work showed that after testing 318 bacterial strains isolated from the GI tract of rainbow trout, two candidate probiotics (C6-6 and C6-8) could inhibit *F. psychrophilum in vitro* and reduced mortality from CWD when used as live microbial feed additives. This has been shown repeatedly in the lab, but more importantly field trials have confirmed this under production conditions. Both probiotic strains have been identified as different *Enterobacter spp.* and genetic sequencing shows they are closely related to *E. amnigenus*, a common ubiquitous soil and aquatic bacteria.

   Following on from this early work, incubation funding this eighteen months allowed us to address a number of practical questions. We found that large scale production of our strains is not a limitation, and that it is possible to lyophilize and store the product for extended periods. Current experiments will tell us if our strains (C6-6 and C6-8) are best delivered alone or in combination. Finally, recent work in our lab demonstrated that both strains are capable of inhibiting *F. columnare*, a significant pathogen of both warmwater coldwater fish species. Results are exciting and clearly demonstrate the commercial potential of these probiotics for aquaculture.

3. List the number of faculty and student participants as a result of funding:

   - David Burbank/Tyson Fehringer (Master's students)
   - Kenneth Cain (Project director – Faculty)

4. What are the potential economic benefits:

   There are a number of potential impacts to Idaho's economy that commercialization of this product could provide. In the private sector, aquaculture represents a major industry in Idaho and supplies approximately 80% of the commercially produced rainbow trout in the US. Disease related impacts affect over 30% of their production on average. The ability to reduce such impacts, even by a small margin, would translate to a direct economic benefit and greater revenue for Idaho companies. In addition, this product would be widely used by public sector (State, Federal, and Tribal) aquaculture facilities rearing trout and salmon for Sportfishing and/or recovery of endangered/threatened stocks. Again, reduced cost of production for State hatcheries and increased opportunities of Sportfishing harvest of trout and salmon would have direct benefit for Idaho and its citizens. Public and private sector aquaculture represents a large potential market and creation of a new start-up company in Idaho would directly benefit Idaho's economy and create jobs.

5. Description future plans for project continuation or expansion:

   We plan to continue work on this product and have submitted a HERC proposal for this current year that will be critical to completing needed studies and expanding our work to testing other important aquaculture species (e.g. catfish and tilapia). A continuation is essential fund the current Master's student (Tyson Fehringer) to completion. We have established potential commercialization partners who would provide expertise and assistance to move the product through the regulatory process.

6. Please provide a final expenditure report (attached) and include any comments here:

   See attached.

7. List invention disclosures, patent, copyright and PVP applications filed, technology licenses/options signed, start-up businesses created, and industry involvement:

   1. Discovery of specific probiotics bacterial strains capable of reducing disease related mortality in aquaculture – Invention Disclosure filed 1/4/2011

   2. Probiotic bacterial strains and method of use to decrease mortality in fish due to bacterial disease. PCT/US12/29896 - filed on April 5th, 2012 by the UI.

8. Any other pertinent information:

FINAL EXPENDITURE REPORT

A. FACULTY AND STAFF		
Name/Title	$ Amount Requested	Actual $ Spent
Kenneth Cain	8,100	0
B. VISITING PROFESSORS		
Name/Title	$ Amount Requested	Actual $ Spent
C. POST DOCTORAL ASSOCIATES/OTHER PROFESSIONALS		
Name/Title	$ Amount Requested	Actual $ Spent
D. GRADUATE/UNDERGRADUATE STUDENTS		
Name/Title	$ Amount Requested	Actual $ Spent
David Burbank/Tyson Fehringer (Master's Students)	9,000	20,151
E. FRINGE BENEFITS		
Rate of Fringe (%) 5.4%	$ Amount Requested	Actual $ Spent
	1,900	1,082
PERSONNEL SUBTOTAL:	**19,000**	**21,233**
F. EQUIPMENT: (List each item with a cost in excess of $1000)		
Item/Description	$ Amount Requested	Actual $ Spent
1.		
2.		
3.		
4.		
EQUIPMENT SUBTOTAL:		
G. TRAVEL		
Description	$ Amount Requested	Actual $ Spent
1. Aquaculture Meetings	3,000	4,038
2.		
3		
TRAVEL SUBTOTAL:	**3,000**	**4,038**
H. PARTICIPANT SUPPORT COSTS:		
Description	$ Amount Requested	Actual $ Spent

1.			
2.			
3			
PARTICIPANT SUPPORT COSTS SUBTOTAL:			
I. OTHER DIRECT COSTS:			
Description		$ Amount Requested	Actual $ Spent
1. Materials and Supplies, tank rental, etc.		11,800	8,417
2.			
3.			
OTHER DIRECT COSTS SUBTOTAL:		11,800	8,417
TOTAL COSTS (Add Subtotals):		33,800	33,688
TOTAL AMOUNT REQUESTED:			33,800
TOTAL AMOUNT SPENT:			**33,688****

**** Remaining balance not posted at current time.