I. Report Title: Bioconversion of Mackeral Byproducts Into Value-Added Products for the Nursery and Plant Propagation Industry

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Grant Number: 98-NER-005 (NA86FD0107)

Date: February 08, 2000

II. Abstract:

This Saltonstall-Kennedy Project 98-NER-105 (NA86FD0107) has helped to develop mackeral hydrolysates, both soluble and insoluble into value-added products that can be commercialized into the nursery and seed industries. This successful NOAA-SK project has resulted in 3 manuscripts published or accepted for publication (Andarwulan and Shetty, 1999a; 1999b; 2000) and 2 more in preparation. In addition, our other previous work in the last 3 years comprising of 3 refereed publications (Eguchi et a1.,1997; Milazzo et al., 1999; Eguchi et al., 1999) and 2 patents (US Patent # 5,906,941 and US patent # 5,882,641) has provided a strong foundation for value-added applications of fishery byproducts in general into the nursery and seed industries. Our work from this NOAA project has clearly identified that the proline, proline analog (hydroxy proline) and proline precursors (glutamic acid and arginine) in the fishery hydrolysates are involved in improving plant propagation efficiency and seed vigor beyond the general nutrient value of fishery byproducts (Milazzo et al., 1999; Andarwulan and Shetty, 1999). This efficiency can be improved with some synergistic phenolics (Andarwulan and Shetty, 1999). Based on this we have preliminary results that elite clonal extracts of oregano with natural phenolics can show improved seed vigor and improved propagation efficiency with fishery hydrolysates (McCue and Shetty, 2000-In preparation). Based on the above solid and very successful conceptual foundation we are proposing a strategy to commercialize mackeral and other fishery byproducts into the nursery and seed industries. In order to accomplish this we have brought together a fishery byproduct producer, Connolly Sea Foods and the University of Massachusetts, spin-off company (PhytoBioSystems) to develop a commercial formulation that can marketed into the US nursery and seed industries.

III. Executive Summary:

This project has helped to develop mackeral hydrolysates into several value-added products for the nursery and propagation industries. The volume of mackeral hydrolysate used is small but it paves the way for development of low volume-high value use of mackeral hydrolysate that could be commercially very valuable and could be extended to other fish waste byproducts and under utilized species. We are also continuing our studies on the high volume-medium value use of mackeral hydrolysates by combining the fertilization benefits with novel growth regulation benefits based on proline metabolism. This is particularly being currently targeted for greenhouse production of herbs and development of potting mix from unhydrolysed debris for herbs and vegetable/nursery crops. Some specfic succeses are stimulation of acclimation response of herb tissue cultures in response to mackeral hydrolysates in combination with acetyl salicylic acid. This work has been published after peer-review in the journal *J. Food Biochemistry*, 23:619-613. A sceond success was the stimulation of phenolic synthesis in anise root cultures by mackeral hydrolysates. This work has been accepted for publication in the journal *Food Biotechnology*. A third success is the stimulation of pea seed vigor by mackeral hydrolysates. This work has been published in the journal *Process Biochemistry*, 35, 159-165. A fourth success is the stimulatory effect of mackeral hydrolysates on soybean shoot organogenesis. This work is being written up for publication for submission to *Process Biochemistry*. Based on previous studies on

fish hydrolysate-apple pomace studies as substrate for growing microbial inoculants (see enclosed patent, US patent # 5,882,641) we are growing several fungi and bacteria on similar substrate for agricultural and environmental applications. We are continuing this work and would require additional time to bring towards the commercialization direction. Another potential application for mackeral hydrolysates is the stimulation of microbial load in petroleum contaminated sites. Preliminary results indicate a 25 ml of standardized mackeral hydrolysates can stimulate bacterial load, particularly *Pseudomonas* in petroleum contaminated soil in 50-100 g soil BIO-BOXES. This was additional work that will take more time to bring it towards commercialization. Another exciting commercial success is the stimulatory effect of mackeral hydrolysates on raspberry clonal production in commercial setting with Nourse Farms of Whatley, MA. Based on these results we are confident that we will develop a commercial formulation in 8-12 months. We are in the initial stages of the project to use modified mackeral hydrolysates nutrient mix for organic flower production.

IV. Purpose:

A. Description of the Problem

The Northeast region's fishery industry is seriously affected by the severe depletion of fish populations of popular bottom-feeding species like cod, haddock and halibut. In order to bring back economic vitality, ecological balance and long-term sustainability of fishery resources, underutilized species are being targeted for use as food and for production of value-added products. Among various underutilized species, mackerel holds much promise for food utilization and conversion into value-added products. Deep-skinned mackerel are being developed into products like nugget supplements and other mincemeat products. Canned, roasted mackerel is popular in the AsianAmerican community and Asian-Pacific countries. Mackerel also has been converted to surimi with good gelling characteristics (Kelleher et al, 1992; 1994) and such products have export potential. In addition to the value-added food uses, mackerel and byproducts from mackerel processing (wastes) were converted to a variety of value-added products that can be used by other sectors of the New England resource industry. Among various approaches, conversion of mackerel and mackerel processing waste into protein hydrolysates for plant propagation and seed industries is very promising based on the success of this project. The remaining insoluble hydrolysates and waste residue can also be developed as growth substrate and carriers for microbial bioinoculants for use in nursery, greenhouse and vegetable production industries and additional studies are required in this regard. Therefore, a novel value-added strategy to use all components of mackerel has been be developed. Such a technological strategy will have economic benefits for the regions fishery industry and can reduce the environmental problems associated with disposal of fish processing wastes of all types.

This Saltonstall-Kennedy Project 98-NER-105 (NA86FD0107) has helped to develop mackeral hydrolysates, both soluble and insoluble into value-added products that can be commercialized into the nursery and seed industries. This successful NOAA-SK grant has resulted in 2 manuscripts accepted for publication (Andarwulan and Shetty, 1999a; 1999b), one in review, and 2 in preparation. In addition, our other previous work in the last 3 years comprising of 3 refereed publications (Eguchi et al., 1997; Milazzo et al., 1999; Eguchi et al., 1999) and 2 patents (US Patent # 5,906,941 and US patent # 5,882,641) has provided a strong foundation for value-added applications of fishery byproducts in general into the nursery and seed industries. Our work has clearly identified that the proline, proline analog (hydroxy proline), and proline precursors (glutamic acid and arginine) in the fishery hydrolysates are involved in improving plant propagation efficiency and seed vigor beyond the general nutrient value of fishery byproducts (Milazzo et al., 1999; Andarwulan and Shetty, 1999). This efficiency can be improved with some synergistic phenolics (Andarwulan and Shetty, 1999). Based on this we have preliminary results that elite clonal extracts of oregano with natural phenolics can show improved seed vigor and improved propagation

efficiency with fishery hydrolysates (McCue and Shetty, 2000-In preparation). Based on the above solid and very successful conceptual foundation we are proposing a strategy to commercialize mackeral and other fishery byproducts into the nursery and seed industries. In order to accomplish this we have brought together a fishery byproduct producer, Connolly Sea Foods and the University of Massachusetts spin-off company PhytoBioSystems to develop a commercial formulation that can marketed into the U.S. nursery and seed industries.

B. Objectives of the Project

The goal of this project was to incorporate mackerel waste hydrolysates into: (1) growth medium for efficient clonal propagation of high value nursery and horticultural crops, and (2) microbial inoculant formulation that will stimulate growth and yield of various nursery and horticultural crops. The success of this project has resulted in an immediate value-added technology that will result in the utilization of all components of mackerel wastes, including unhydrolyzed debris and bone chips. Such a value-added technology will be economically linked to $4 billion U.S. nursery and plant propagation industry. It will also substantially reduce the environmental effects associated with fishery wastes. The overall goal of the technology that was proposed was to provide substantial economic and environmental benefits to the Northeast Fishery Industry through development of novel value-added products.

VI. Approach:

A. Detailed Description of the Work That Was Performed

The focus of the project as defined under the objectives was to develop several value-added products using mackeral hydrolysates for the nursery and propagation industry. The approach and methods followed are outlined below and the details are covered under the accomplishment section below. More details are available from the manscripts that are attached to this report.

We used the ready made mackeral hydrolysates produced in collaboration with Conolly Sea Foods of Gloucestor, MA. These hydrolysates were used in various tissue culture mediums and seed treatment formulas that led to specific accomplishments outlined below. In each case painstaking tissue culture studies and specific metabolite analysis to understand the effect of mackeral hydrolysates were undertaken. Metabolic studies also included the effect on key regulatory enzymes in the diverse plant systems. Microbial inoculants were also made from unhydrolysed debris and bone wastes using a ration of 1 part of hydrolysates and 10 parts of peat or apple pomace. Inoculants like *Rhizobium, Tdchoderma, Azospirilum, Bacillus and Pseudomunas* were successfully grown and the effect on plant growth and development assessed based on metabolite and enzyme analysis. These results are incomplete and need more time for commercial development. The most successful part of the work the improved propagation of commerial raspberry at Nourse Farms using mackeral hydrolysates. Hydrolysates were applied to both tissue culture medium as well as to irrigation water during transplantation at the rate of 10 ml concentrate/liter. More description of the methods can be obtained from the manuscripts published as a result of this research.

B. Project Management:

This project was directed by Professor Kalidas Shetty, who was the principal investigator. In addition to working out the details and managing the project he developed the key tissue culture methods for the project. In the first 8 months of the project the key experiments were undertaken by Mss. Nuri Andarwulan. Her dedication and effort in this project led to several manuscripts in peer-reviewed journals. After 8 months the second half of the project between 9-18 months was undertaken by a series of graduate student assistants who pursued different aspects of the specific projects

reported here. These assistants were Patrick McCue, Howard Webley, Sarah Stycyraz, Preethi Shetty, Zuoxing Zheng and Huynah Pham. As a result of these efforts we were able to explore a few more areas than originally planned. The raspberry project at Nourse Farms were managed by Susan Cheplick at Nourse Farms, but she did not receive any financial support from this project.

C. Actual Accomplishments and Findings:

This project has helped to develop mackeral hydrolysates into several value-added products for the nursery and propagation industries. The volume of mackeral hydrolysate used was small but it paves the way for development of low volume-high value use of mackeral hydrolysate that could be commercially very valuable and could be extended to other fish waste byproducts and under utilized species. We are also continuing our studies on the high volume-medium value use of mackeral hydrolysates by combining the fertilization benefits with novel growth regulation benefits based on proline metabolism. This is particularly being currently targeted for greenhouse production of herbs and development of potting mix from unhydrolysed debris for herbs and vegetable/nursery crops. Some specfic succeses are :

Project 1: Stimulation of acclimation response of herb tissue cultures in response to mackeral hydrolysates in combination with acetyl salicylic acid. This study shows the stimulation of phenolic antioxidant, rosmarinic acid and lignification in oregano clonal line O-1 and O-5 in response to mackeral hydrolysate, acetyl salicylic acid and combinations of both. Mackeral hydrolydates in combination with acetyl salicylic acid stimulated rosmarinic acid and total phenolic contents. This work has been published after peer-review in the journal *J. Food Biochemistry*, 23:619-613.

Project 2: (Stimulation of phenolic synthesis in anise root cultures by mackeral hydrolysates.) Our laboratory has recently published a manuscript to *J. Agricultural and Food Chemistry* (Vo1:47:1776-1780{1999) showing the naturally transformed root cultures of anise produce a seed germination and synchronization phenolic metabolite. This metabolite epoxypseudoisoeugenol-2-methylbutyrate (EPB) is produced from anethole, a phenolic metabolite found in anise seeds. This present work dearly shows, compared to any other treatment, including other hydrolysates and aspirin, mackeral hydrolysate alone at 1000-2000 mg/L significantly stimulated EPB in anise root cultures. This work has been accepted for publication in the journal *Food Biotechnology*.

Project 3: Stimulation of pea seed vigor by mackeral hydrolysates. Mackeral hydrolysates stimulated vigor of germinating seeds as measured by growth (height and weight). Further, the phenolic and guiaicol peroxidase activity were high, indicating higher lignification linked to enhanced growth and vigor. Glucose 6-phosphate dehydrogenase activity and free proline content were concurrently stimulated during early stages (day 5) indicating that mackeral hydrolysate-linked phenolic stimulation linked to pea vigor response may be tied to proline-linked pentose phosphate pathway. The optimum mackeral hydrolysates required for the desired effect on pea vigor was 1000-2000 mg/L. This work has been published in the journal *Process Biochemistry*, 35, 159-165.

Project 4: (Effect of mackeral hydrolysates on stimulation of soybean shoot organogenesis). Our investigations dearly showed that mackeral hydrolysates were superior to yeast, bactopeptone, casein, and soy hydrolysates in stimulating in vitro shoot organogenesis from hypocotyls of soybean. This work is being written up for publication for submission to *Process Biochemistry*.

Project 5: (Production of microbial inoculants). Based on previous studies on fish hydrolysate-apple pomace studies as substrate for growing microbial inoculants (U.S. patent # 5,882,641) we are growing several fungi and bacteria on similar substrate for agricultural and environmental applications. We are continuing this work and would require additional time to bring towards the commercialization direction.

Project 6: (Stimulation of microbial load in petroleum contaminated sites with mackeral hydrolysates). Preliminary results indicate a 25 ml of standardized mackeral hydrolysates can stimulate bacterial load, particularly *Pseudomonas* in petroleum contaminated soil in 50 100-g soil BIO-BOXES. We want to determine if these wastes are degraded as a result. This has implications for using mackeral and other fish hydrolysates for pollution clean-up. This was additional work that will take more time to bring it towards commercialization.

Project 7: Production of *Trichoderma* inoculants and effect on seed vigor in peas. Based on previous studies on fish hydrolysate-apple pomace studies as substrate for growing microbial inoculants we are growing several fungi and bacteria on similar substrate for agricultural and environmental applications. The results of these efforts show dearly that *Trlchiderma* inoculants made using fish hydrolysates are superior to those made from ammonium nitrate. These inoculants stimulate seed vigor in peas. This work is on-going and we are in the process of determining whether proline metabolism is activated due to the mackeral hydrolysate that is used for the inoculant production. This inoculant concept when completely proved can be commercially developed within the next 6 months of further research on wide variety of plant species.

Project 8: Production of *Rhizobium* inoculants for legumes. We have successfully produced *Rhizobium* inoculants with unhydrolysed mackeral waste with bone chips in both apple pomace and peat moss medium. Our investigation on the effect of such inoculants are delayed due to the logistics of the greenhouse facilities. We are now testing under gro-lights in the laboratory conditions and also making arrangements to test in local commercial greenhouse facilities in Amherst and Whatley.

Project 9: Production of *Azotobacter* and *Bacillus polymyxa* inoculants for vegetable production. Like *Rhizobium* inoculants we have successfully grown *Azotobacter* and *Bacillus polymyxa* bacterial inoculants in unhydrolysed mackeral waste in both apple pomace and peat moss medium. These inoculants have not been completely tested. We are evaluating it use for local crops like tomato, chilli peppers, egg plant and potato (All in the Solanaceae family). There are some preliminary evidence of it value for cucumber and melons but the results are not conclusive and must be repeated to develop a commercial formulation.

Project 10: Effect of mackeral hydrolysate on raspberry clonal production in commercial setting with Nourse Farms of Whatley, MA. This has been the most exciting part of our work so far. A success in a commercial setting. We developed a modified mackeral hydrolysate with phenolic extracts from oregano clonal line O-1. This modified mackeral hydrolysate was very effective in improving clonal vigor, growth and development of a raspberry variety (Jewel) that Nourse Farms were having a lot of difficulty propagating in the field conditions. The improvements are very evident. We are now developing a systematic protocol with Nourse Farms to test this on a larger scale on other small fruits, strawberry and blackberry. Based on these results we are confident that we will develop a commercial formulation in 8-12 months.

Project 11: Modified mackeral hydrolysate nutrient mix for organic flower production. We are in the early stages of developing a mackeral hydrolysate nutrient mix to improve flowering in ornamental species. We are also continuing our studies on the high volumemedium value use of mackeral hydrolysates by combining the fertilization benefits with novel growth regulation benefits as above. This is particularly being currently targeted for greenhouse production of herbs and fungal and bacterial bioinoculants for herbs and vegetable/nursery crops. We have been very successful in improving propagation of raspberry clones for a commercial entity in Massachusetts, Nourse Farms of Whatley, MA.

D. Problems:

We have not completed this project to the best of our satisfaction from the point of view of commercialization. We had some problems obtaining greenhouse facilities for

the project at the University of Massachusetts. We have this support now from Nourse Farms of Whatley, MA and therefore are pursueing the commercialization direction further. The Bioinoculant efforts also need more time, efforts, and resources for completion to bring it to a commercial success.

E. Future Need:

The projects needs another 12-18 months of support and effort to realize its full potential. We are pursuing this need both from additional funding from NOAA as well as private industry sources.

VII. Evaluation:

In general the goals of the projects were attained. Through this project we have fully realized the value of mackeral hydrolysates for value-added applications relevant to nursery and propagation industry. We were able to explore both the plant and bioinoculant applications fully. In addition there is promise that mackeral and other fishery hydrolysates could have potential to enhance pollution clean-up like from petroleum wastes. The commercial potential of the above projects have been not fully realized. There needs to be additional support for more greenhouse and field evaluation and development of commercial formulations following thorough testing. Complete greenhouse evaluations were not completed partly due to difficulty in getting space at UMASS. This needs more support from both interested industry sources as well as NOAA.

No modifications were made to the general directions of the objectives. When we ran into difficulties with greenhouse space for evaluations we explored several additional applications of the concepts on the use of mackeral hydrolysates. Therefore, in the long-run this could prove to have very good commercial prospects. On the positive side we are pleased that we were able to hire many very good student assistants who helped to explore more applications of value.

VIII. Dissemination of Project Results:

Peer-reviewed publications is the major route of dissemination of project results. The currently published manusrcipts are attached to this report. Reprints of published papers and even pre-prints will be freely available to the public and fishing industry interests. We have had many requests in this regard and have readily provided the information to those who request them.