

Source: **UNIV OF CONNECTICUT** submitted to **CRIS**

PRODUCTION FOR SUPERIOR RAINBOW TROUT BROODSTOCKS BY GENETIC MANIPULATION

Sponsoring Institution	Agricultural Research Service/USDA	Project Status	TERMINATED
		Funding Source	USDA COOPERATIVE AGREEMENT
Reporting Frequency	Annual	Accession No.	0409543
Grant No.	(N/A)	Project No.	1930-31000-011-01S
Proposal No.	(N/A)	Multistate No.	(N/A)
Program Code	(N/A)	Project Start Date	May 15, 2005
Project End Date	May 14, 2010	Grant Year	(N/A)

Project Director
REXRoad III C E

Recipient Organization
UNIV OF CONNECTICUT
(N/A)
STORRS,CT 06269

Performing Department
(N/A)

Non Technical Summary
(N/A)

Animal Health Component 50%

Research Effort Categories

Basic 0%
Applied 50%
Developmental 50%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
307	3711	1080	100%

Knowledge Area
307 - Animal Management Systems;

Subject Of Investigation
3711 - Trout;

Field Of Science
1080 - Genetics;

Keywords

rainbow	broodstocks	lines	trout
disease	resistance	muscle	growth
mstn	gene		

Goals / Objectives

The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true-breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene.

Project Methods

Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true-breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, reducing the costs for care, feeding and rearing space.

Progress 10/01/10 to 09/30/11

Outputs

Progress Report Objectives (from AD-416) The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true-breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene. Approach (from AD-416) Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true-breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, reducing the costs for care, feeding and rearing space. With the rapid growth of the human population in the world and the increased consumption of fishery products for health reasons, the world demand of fishery products is mounting rapidly. To this, many countries have turned to various forms of intensive aquaculture. However, the rapid escalation of fish production by aquaculture has resulted in several negative impacts such as disease outbreaks in aquaculture facilities. These negative impacts have brought about significant economic losses in the aquaculture industry. A solution to some of these problems is the development of disease resistant fish species by genetic engineering. This project focused on development of rainbow trout strains that are resistant to infection by bacterial and viral pathogens by introducing antimicrobial peptide genes through genetic engineering. Transgenes containing pig cecropin P1 and synthetic cecropin-like (CF-17) genes were introduced into rainbow trout via a sperm mediated gene transfer method. The P1 generation transgenic animals were crossed with non-transgenic fish to establish F1 founder transgenic families. Totally 20 families of cecropin P1 and 10 families of CF-17 transgenic fish were established. Each of these transgenic families was crossed to non-transgenic fish to generate F2, F3 and F4 transgenic offspring and the fingerlings of these fish were challenged with *Aeromonas salmonicida* (an important bacterial pathogen for rainbow trout) and infectious hematopoietic necrosis virus (IHNV). Repeated challenge studies revealed that a total of nine families of cecropin P1 transgenic fish and five families of CF-17 transgenic fish exhibited the characteristic of resistance to *Aeromonas salmonicida* and IHNV. Each of these families was bred into all male homozygous state and the sperm samples from all families were cryo-preserved. Although we have attempted to produce rainbow trout strains by introducing siRNA transgenes to down-regulate the expression of myostatin gene many times, we failed to produce any viable transgenic trout. The ADODR is in frequent contact with the cooperator through phone calls, email, and site visits in addition to receipt of written reports.

Impacts

(N/A)

Publications

Progress 10/01/09 to 09/30/10

Outputs

Progress Report Objectives (from AD-416) The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true- breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene. Approach (from AD-416) Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true- breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, reducing the costs for care, feeding and rearing space. Using the technology of androgenesis, the following homozygous clonal lines of transgenic fish containing cecropin P1 constructs were created: S7-342-F695, S8-505-G231, S7-375-F180, S9746-F509 and S9-659-F-073 which are resistant to IHNV; and *A. salmonicida*, and S9-638-F297, U6-768-G410, A12-944 and A13-831 which are resistant to IHNV alone. Through genotyping and sex identification by PCR analysis, all male individual homozygous fish have been tagged. Individuals from families S7#375-F-073 and S9#659-F-180 have reached reproductive maturation. The other families will reach reproductive maturation by December 2010. The expression of cecropin P1 transgene in heart, spleen, muscle and liver of individual fish from S7#375-F-073 and S9#659-F-180 have been confirmed by RT-PCR analysis. Sperm samples from S7#375-F-073 and S9#659-F-180 homozygous transgenic fish families have been cryopreserved. Sperm of S7#375-F-073 and S9#659-F-180 were out-crossed to eggs from non-transgenic fish and the resulting offspring were challenged with *Aeromonas salmonicida*. Results of the challenge studies showed that progeny derived from both families of fish exhibited resistant characteristics to *Aeromonas salmonicida*. A construct which contains *crtW* and *crtZ* genes driven by the Beta-actin gene promoter has been constructed. This double transgene construct is transfected into CHSE cells (Chinook salmon embryonic cells) to test for conversion of Beta-carotene into astaxanthin by HPLC analysis. The transgene has been proven to be functional. The transgene insert will be introduced into rainbow trout via electroporating the sperm following conditions developed in our laboratory. The resulting fish will be raised to adulthood for identification of the presence of transgene by PCR analysis. The ADODR is in frequent contact with the cooperator through phone calls, email, and site visits in addition to receipt of written reports.

Impacts

(N/A)

Publications

Progress 05/15/05 to 05/14/10

Outputs

Progress Report Objectives (from AD-416): The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true- breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene. Approach (from AD-416): Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true- breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, reducing the costs for care, feeding and rearing space. Project is awaiting Administrative close-out. Final

Report was submitted through the FY 2011 Annual Reports process.

Impacts

(N/A)

Publications

Progress 10/01/08 to 09/30/09

Outputs

Progress Report Objectives (from AD-416) The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true- breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene. Approach (from AD-416) Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true- breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, s reducing the costs for care, feeding and rearing space. Significant Activities that Support Special Target Populations We have conducted challenge studies on transgenic fish with the bacterial pathogen *A. salmonicida* and the viral pathogen IHNV. Results of the challenge studies showed fish of these transgenic families consistently exhibited resistant characteristics to *A. salmonicida* and IHNV. Sperm samples from these families have been cryopreserved. Furthermore, we initiated work to breed fish homozygous for the transgene by androgenesis. To date, we have successfully bred two cecropin P1 transgenic fish families; although androgenesis has also been conducted on the remaining 7 cecropin P1 transgenic fish families, the resulting fish are too small to be sampled for genotyping at this moment. Fin tissue samples will be collected from these fish for genotyping by this fall and we expect to complete genotyping these fish by December 2009. The ADODR is in frequent contact with the cooperator through telephone calls, email, and annual site visits in addition to receipt of written reports.

Impacts

(N/A)

Publications

- Chiou, P.P., Khoo, J., Chun, C., Chen, T.T. 2005. Transgenic Fish. In: Meyers, R., editor. Encyclopedia of Molecular Cell Biology and Molecular Medicine. 2nd Edition, Vol.14. Federal Republic of Germany: VCH Verlagsgesellschaft mbH. p. 473-503.
- Chen, T.T., Chen, M.J., Chiou, T., Lu, J.K. 2009. Transfer of foreign DNA into aquatic animals by electroporation. In: Nakamura, H., editor. Electroporation and Sonoporation in Developmental Biology. Tokyo, Japan: Springer. p. 229-237.
- Lo, J.H., Chiou, P.P., Chen, T.T. 2007. Molecular cloning and expression analysis of rainbow trout (*Oncorhynchus mykiss*) CCAAT/enhancer binding protein genes and their responses to induction by GH in vitro and in vivo. *Journal of Endocrinology*. 194:393-406.
- Chiou, P.P., Khoo, J., Bols, N.C., Douglas, S., Chen, T.T. 2005. Effects of linear cationic α -helical antimicrobial peptides on immune-relevant genes in trout macrophages. *Developmental and Comparative Immunology*. 30:797-806.
- Chiou, P.P., Lin, C., Bols, N.C., Chen, T.T. 2007. Characterization of virus/double-stranded RNA-dependent induction of antimicrobial peptide hepcidin in trout macrophages. *Developmental and Comparative Immunology*. 31:1297-1309.
- Chen Hung-Chih, M., Li, Y., Chang, Y., Wu, S., Gong, H., Lin, G., Chen, T. T., Wu, J. 2006. Co-induction of hepatic IGF-I and progranulin mRNA by growth hormone in tilapia, *Oreochromis mossambicus*. *General and Comparative Endocrinology*. 150:212-218.
- Sallum, U.W., Chen, T.T. 2008. Inducible Resistance of Fish Bacterial Pathogens to the Antimicrobial Peptide Cecropin B. *Antimicrobial Agents and Chemotherapy*. 52(9):3006-3012.

Progress 10/01/07 to 09/30/08

Outputs

Progress Report Objectives (from AD-416) The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true- breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene. Approach (from AD-416) Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true- breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, reducing the costs for care, feeding and rearing space. Significant Activities that Support Special Target Populations Several lines of fish with targeted transgenes for disease resistance have reached their third generation and were shown to have enhanced resistance to both bacterial (*Aeromonas salmonicida*) and viral (IHNV) pathogens. Studies also indicated the gene cecropin B could serve as an adjuvant to improve DNA vaccination. Monitoring was accomplished via telephone discussions and periodic written reports. National Program 106, Component 3, Genetic Improvement.

Impacts

(N/A)

Publications**Progress** 10/01/06 to 09/30/07**Outputs**

Progress Report Objectives (from AD-416) The objectives of this cooperative research project are to: 1) Complete the development of rainbow trout breeding stocks that consist of true- breeding individuals for the cecropin gene, a gene which increases disease resistance; 2) Complete the identification and characterization of genes in rainbow trout macrophage cells that are responsive to induction by cecropin B, and; 3) Develop rainbow trout broodstock for aquaculture with enhanced muscle growth by down-regulating the MSTN-1 gene. Approach (from AD-416) Bacterial and viral pathogens will be used to challenge F2 transgenic fish and those that exhibit resistance to one or more of the pathogens will be bred together as the initial stages for development of a true- breeding stock. In addition, the production of gynogens from the eggs of resistant animals will be attempted to shorten the time to make the genes completely homozygous. Rainbow trout macrophages will be treated with cecropins and the expression of genes will be monitored by the use of cDNA subtraction hybridization. This technique will allow the identification of the genes which play a role in the adaptive immune response in rainbow trout. Finally, the development of rainbow trout broodstock with increased muscle growth will be attempted by use of the RNAi gene constructs to down-regulate the MSTN gene. Preliminary results suggest that this approach will lead to strains with enhanced somatic growth rate, reducing the costs for care, feeding and rearing space. Significant Activities that Support Special Target Populations This report serves to document research conducted under a specific cooperative agreement between ARS and the University of Connecticut. In FY 2007 this project has progressed to the second generation in several additional lines with targeted transgenes that appear to have enhanced disease resistance. Other groups of transgenic rainbow trout have been bred to the F2 generation. Monitoring was accomplished via telephone discussions and periodic written reports.

Impacts

(N/A)

Publications**Progress** 10/01/05 to 09/30/06**Outputs**

Progress Report 1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter? Rainbow trout is an important aquaculture fish species both for its commercial value as a food fish as well as for its recreational value to

fishermen in the northern United States and Canada. A major handicap of large-scale culturing of this fish species is disease outbreaks resulting from infection by bacterial, fungal, parasitic and viral pathogens. Development of strains of rainbow trout resistant to pathogen infection via genetic manipulation will be the solution to this problem. To resolve this problem we have initiated work to produce new rainbow trout strains with increased resistance to infection by bacteria, viruses, fungi and parasites by genetic engineering. Furthermore, we have also conducted studies to characterize genes that are responsive to infection by pathogens in rainbow trout in order to develop alternative strategies of disease protection in rainbow trout. The overall goals for the entire project are: (1) Develop rainbow trout brood stocks with disease resistant genetic trait for aquaculture. (2) Identify and characterize the genes responsive to induction by cecropin B in rainbow trout macrophage cells. (3) Develop rainbow trout brood stock with enhanced muscle growth for aquaculture by down-regulating MSTN gene.

2. List by year the currently approved milestones (indicators of research progress) Milestones for FY2005

1. Conduct challenge studies of F2 Rbt-99 fish with a viral pathogen, infectious hematopoietic necrosis virus (IHNV).
2. Characterize F2 transgenic fish with respect to transgene inheritance and transgene expression.
3. Continue to breed fish from F2 Rbt-99 transgenic fish to homozygosity for the cecropin gene.
4. Continue screening genes in trout macrophage cells that are regulated by antimicrobial peptides.
5. Produce RNAi gene constructs and test the feasibility of these constructs in down regulating MSTN-1 gene in a rainbow trout cell line in vitro.

Milestones for FY2006

1. Conduct challenge studies of F3 Rbt-99 and F2 Rbt-02 fish against *A. salmonicida* and IHNV.
2. Initiate breeding of Rbt-99 transgenic fish to homozygous by androgenesis.
3. Using microarray technology complete screening of genes that are regulated by antimicrobial peptide.
4. Complete characterization of cecropin resistant bacterial pathogens for infectivity to hosts.
5. Cryopreserve sperm of F2 Rbt-02 fish.
6. Identify strong muscle-specific promoters from rainbow trout.
7. Determine whether antimicrobial peptide can serve as an adjuvant in vaccination.

Milestones for FY2007

1. F4 Rbt-99 challenge studies.
2. Breed Rbt02 to homozygous.
3. Cryopreserve F3 Rbt-99 sperm and F2-Rbt-02 sperm
4. Complete the genotyping of homozygous Rbt99 fish.
5. Constructing and testing RNAi for MSTN
6. Breeding homozygous Rbt-02 fish
7. Confirm microarray analysis results by real-time PCR
8. Publish results of research with Rbt99 transgenic fish.

Milestones for FY2008

1. Conduct scale-up challenge studies with homozygous Rbt99 fish.
2. Conduct F3 Rbt-02 challenge studies.
3. Cryopreservation of F3 Rbt-02 sperm.
4. Genotyping homozygous Rbt-02 fish.
5. Produce P1 transgenic fish carrying RNAi for MSTN.

Milestones for FY2009

1. Genotyping P1 transgenic fish carrying RNAi for MSTN.
2. Conduct scale-up challenge studies with homozygous Rbt-02 fish
3. Cryopreserve sperm samples of homozygous Rbt-99 and Rbt-02 fish
4. Comparing the expression patterns of immune responsive genes in transgenic fish and non-transgenic fish.

Milestones for FY2010

1. Establish F1 family of transgenic fish carrying RNAi of MSTN.
2. Re-confirm the results of scale-up challenge studies with newly established animals by crossing homozygous transgenic fish (i.e., Rbt-99 and Rbt-02) with non-transgenic fish.
3. Prepare manuscripts reporting results of challenge studies with Rbt02 fish; and the expression patterns of immune responsive genes in transgenic fish and non-transgenic fish.

4a List the single most significant research accomplishment during FY 2006. Another anti-microbial peptide found that is induced in rainbow trout. We have shown for the first time that another antimicrobial peptide, hepcidin, in rainbow trout could be induced by not only bacterial infection but also by synthetic poly-inosinic-poly-cytidylic acid (poly- I:C), a mimic of viral double-stranded (ds) RNA. The induction of hepcidin in fish by bacterial and viral infections may be important in protecting the animal from secondary bacterial infection by either directly killing the bacteria or restraining free iron level in the serum.

4b List other significant research accomplishment(s), if any. Cecropin transgenic fish exhibit resistance to a viral pathogen IHNV: We have confirmed that two subfamilies of S7 founder family (i.e., S7#342- F695 and S2#375-F180), two subfamilies of S8 founder family (S8#505-G275 and S8#505-G231), four subfamilies of S9 founder family (S9#638-F297, S9#659-F073, S9#746-F509 and S9#659-541) are resistant to *A. salmonicida* and IHNV. We have also confirmed that another four families Rbt-99B founder fish are resistant to IHNV. Some bacterial pathogens may develop resistant to antimicrobial peptides: We have demonstrated conclusively that some bacterial pathogens can gain resistance to antimicrobial peptide via physiological adaptation, but will return to non-resistant status if the selection pressure is removed. Furthermore, the resistant pathogens do not exhibit higher pathogenicity than the non-resistant counterparts.

4d Progress report. This report serves to document research conducted under a Specific Cooperative Agreement between ARS and the University of Connecticut. Additional details of research can be found in the report for the parent CRIS 1930-31000-007, Utilizing Genetics for Enhancing Cool and Cold Water Aquaculture Production.

5. Describe the major accomplishments to date and their predicted or actual impact. Annual loss of fish as a consequence of infection by bacterial and viral pathogens impacts the productivity of aquaculture of trout and other finfish/shellfish tremendously in the United States. There is an urgent need to develop strategies to overcome this problem. Through our earlier studies, it became clear that manipulation of antimicrobial peptide genes in trout may result in development of new strains of fish that are resistant to bacterial and viral infection. We have demonstrated that the gene products of cecropin and CF-17 genes can effectively kill fish bacterial and viral pathogens. By introducing cecropin and CF-17 genes into rainbow trout embryos, we have produced transgenic trout that exhibited strong resistance to bacterial and viral pathogens. In addition, we have obtained evidence indicating that antimicrobial peptides will not result in selecting microorganisms with resistance characteristics to these peptides. Work is underway to breed rainbow trout with homozygous antimicrobial peptide genes for large-

scale grow out. Once the work is completed, the first potential impact of this project is that the brood stocks of these fish could be used by trout farmers for commercialization without the worry of disease outbreaks. The second impact of the project is that the same strategy can be adopted to develop highly disease resistant finfish and crustacean species for aquaculture. With the availability of these transgenic fish, the U.S. could become the leader in providing disease-resistance fish stocks for aquaculture throughout the world. 7. List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below). Chiou, P Peter, Bols, Niels, Douglas, Sue and Chen, Thomas T. 2005. Regulation of Immune-Relevant Genes in the Trout Macrophage Cell Line RTS- 11 by Antimicrobial Peptides. *Developmental and Comparative Immunology* 30: 797-806.

Impacts

(N/A)

Publications

Progress 10/01/04 to 09/30/05

Outputs

1. What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter? Rainbow trout is an important aquaculture fish species both for its commercial value as a food fish As well as for its recreational value to fishermen in the northern United States and Canada. A major handicap of large-scale culturing of this fish species is the disease outbreak resulting from infection by bacterial, fungal, parasitic and viral pathogens. Development of strains of rainbow trout resistant to pathogen infection via genetic manipulation will be the solution to this problem. To resolve this problem we have initiated work to produce new rainbow trout strains with increased resistance to infection by bacteria, viruses, fungi and parasites by genetic engineering. Furthermore, we have also conducted studies to characterize genes that are responsive to infection by pathogens in rainbow trout in order to develop alternative strategies of disease protection in rainbow trout. 2. List the milestones (indicators of progress) from your Project Plan. The overall goals for the entire project are: (1) Develop rainbow trout brood stocks with disease resistant genetic trait for aquaculture. (2) Identify and characterize the genes responsive to induction by cecropin B in rainbow trout macrophage cells. (3) Develop rainbow trout brood stock with enhanced muscle growth for aquaculture by down-regulating MSTN gene. Milestones for FY2005: I. Conduct challenge studies of F2 Rbt99 fish with a viral pathogen, IHN. II. Characterize F2 transgenic fish with respect to transgene inheritance and transgene expression. III. Continue to breed homozygous fish from F2 Rbt-99 transgenic fish to homozygous. IV. Continue screening genes in trout macrophage cells that are regulated by antimicrobial peptides. V. Produce RNAi gene constructs and test the feasibility of these constructs in down regulating MSTN-1 gene in a rainbow trout cell line in vitro. Milestones for FY2006: I. Conduct challenge studies of F3 Rbt99 and F2 Rbt-02 fish against *A. salmonicida* and IHN. II. Initiate breeding of Rbt-99 transgenic fish to homozygous by androgenesis. III. Microarray screening of genes that are regulated by antimicrobial peptide. IV. Complete characterization of cecropin resistant bacterial pathogens for infectivity to hosts. V. Cryopreserve sperm of F2 Rbt02 fish. VI. Identify strong muscle-specific promoters from rainbow trout. VII. Investigate whether antimicrobial peptide can serve as an adjuvant in vaccination. Milestones for FY2007: I. F4 Rbt99 challenge studies. II. Breed Rbt02 to homozygous. III. Cryopreserve F3 Rbt99 sperm and F2 Rbt-02 sperm. IV. Genotyping homozygous Rbt99 fish. V. Constructing and testing RNAi for MSTN VI. Breeding homozygous Rbt02 fish VII. Confirm microarray analysis results by real-time PCR VIII. Publish results of research with Rbt99 transgenic fish. Milestones for FY2008 I. Conduct scale-up challenge studies with homozygous Rbt99 fish. II. Conduct F3 Rbt02 challenge studies. III. Cryopreservation of F3 Rbt02 sperm. IV. Genotyping homozygous Rbt02 fish. V. Produce P1 transgenic fish carrying RNAi for MSTN. Milestones for FY2009: I. Genotyping P1 transgenic fish carrying RNAi for MSTN. II. Conduct scale-up challenge studies with homozygous Rbt02 fish III. Cryopreserve sperm samples of homozygous Rbt99 and Rbt02 fish IV. Comparing the expression patterns of immune responsive genes in transgenic fish and non-transgenic fish. Milestones for FY2010: I. Establish F1 family of transgenic fish carrying RNAi of MSTN. II. Re-confirm the results of scale-up challenge studies with newly established animals by crossing homozygous transgenic fish (i.e., Rbt99 and Rbt02) with non-transgenic fish. III. Prepare manuscripts reporting results of challenge studies with Rbt02 fish; and the expression patterns of immune responsive genes in transgenic fish and non-transgenic fish. 3a List the milestones that were scheduled to be addressed in FY 2005. For each milestone, indicate the status: fully met, substantially met, or not met. If not met, why. 1. Conduct challenge studies of F2 fish derived from Rbt-99 with a viral pathogen, IHN. Milestone Fully Met 2. Characterize F2 transgenic fish with respect to transgene inheritance and transgene expression. Milestone Fully Met 3. Continue to breed some of the Rbt-99 transgenic fish to homozygous. Due to limitation of time, this milestone will be initiated in November 2005. Milestone Substantially Met 4. Continue screening genes in trout macrophage cells that are regulated by antimicrobial peptides. We have completed the milestone for this year. Milestone Fully Met 5. Produce RNAi gene constructs and test the feasibility of these constructs in down regulating MSTN-1 gene in a rainbow trout cell

line in vitro. Milestone Substantially Met 3b List the milestones that you expect to address over the next 3 years (FY 2006, 2007, and 2008). What do you expect to accomplish, year by year, over the next 3 years under each milestone? (1) Year I (2006): I. Conduct challenge studies of F3 Rbt99 fish against *A. salmonicida* and IHNV. II. Conduct challenge studies of F2 Rbt-02 against *A. salmonicida* and IHNV. III. Initiate breeding of Rbt-99 transgenic fish) to homozygous by androgenesis. IV. Microarray screening of genes that are regulated by antimicrobial peptide. V. Complete characterization of cecropin resistant bacterial pathogens for infectivity to hosts. VI. Cryopreserve sperm of F2 Rbt02 fish VII. Identify strong muscle-specific promoters from rainbow trout. VIII. Investigate whether antimicrobial peptide can serve as an adjuvant in vaccination. (2) Year II (2007): I. F4 Rbt99 challenge studies. II. Breed Rbt02 to homozygous. III. Cryopreserve F3 Rbt99 sperm and F2Rbt99 sperm. IV. Genotyping homozygous Rbt99 fish. V. Construct and test RNAi for MSTN VI. Breed homozygous Rbt02 fish VII. Confirm microarray analysis results by real-time PCR. VIII. Publish results of research with Rbt99 transgenic fish (3) Year III (2008): I. Conduct scale-up challenge studies with homozygous Rbt99 fish. II. Conduct F3 Rbt02 challenge studies. III. Cryopreservation of F3 Rbt02 sperm IV. Genotyping homozygous Rbt02 fish. V. Produce P1 transgenic fish carrying RNAi for MSTN 4a What was the single most significant accomplishment this past year? We demonstrated conclusively that cecropin B or CF-17 peptide can induce inflammatory responses in macrophages. The induction of the inflammatory response does not further block the effect of LPS induction of inflammatory response in macrophage cells. These results suggest that cecropin or CF-17 could serve as an adjuvant in fish vaccination. 4b List other significant accomplishments, if any. 1. Cecropin transgenic fish exhibit resistant to a viral pathogen IPNV: We have confirmed that 5 families (S9#746, A12#944, A13#831, Cec230#3255 and S9-A26) of F2 Rbt99 transgenic fish are resistant to the viral pathogen IHNV. 2. F1 transgenic fish with CF-17 transgene have been produced: We have successfully produced 10 families of F1 transgenic fish carrying CF-17 transgene. 3. Cryopreservation of trout sperm: We have successfully cryopreserved sperm from 21 families of Rbt09 transgenic fish. 4. Some bacterial pathogens may develop resistant to antimicrobial peptides: We have demonstrated conclusively that some bacterial, pathogens can gain resistant to antimicrobial peptide via physiological adaption, bur will return to non-resistant status if the selection pressure is removed. 4d Progress report. This report serves to document research conducted under a specific cooperative agreement between ARS and the University of Connecticut. Additional details of research can be found in the report for the parent CRIS 1930-31000-007-00D Utilizing Genetics for Enhancing Cool and Cold Water Aquaculture Production. 5. Describe the major accomplishments over the life of the project, including their predicted or actual impact. (1) We have discovered that transgenic model fish (medaka) carrying cecropin transgene are resistant to infection by bacterial pathogens. (2) Cecropin and the synthetic analog (CF-17) can inhibit the propergation of IHNV and other important fish virus, suggesting that CF- 17 may be an excellent candidate for controlling viral diseases in fish. (3) We have obtained evidence to show that F2 transgenic fish carrying cecropin B transgene exhibited resistant to bacterial and viral pathogens. (4) Although prolonged exposure of Gram-negative fish pathogens to cecropin may result in selection of resistant cells via physiological adaptation, yet these cells possess no infectivity to fish hosts. These results suggest that cecropin is far safer than any commercially available antibiotics. (5) We have provided conclusive evidence that cecropin B or CF-17 possessed pro-inflammatory effect to macrophage, suggesting that cecropin or CF-17 has direct effect to the immune system of the fish. Furthermore, it also suggests that these peptides can serve as an adjuvant in vaccination.

Impacts

(N/A)

Publications