California Sea Grant College Program
Progress/Completion Report

Project Information

Year 2nd Year
NOAA Grant No.: NA04OAR4170038
Number R/A-124
Start Date: 3/1/2005 **Completion Date:** 5/31/2007
Title Understanding the Pathogenesis of Streptococcus iniae Infection in Fish and Development of an Effective Vaccine for Use in Aquaculture

Project Leader

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Project Hypothesis

Project Goals and Objectives

S. iniae infection has emerged as a serious fish health and economic problem in intensive U.S. aquaculture operations. Current antibiotic options are few and possess severe practical limitations and potential adverse environmental impacts. We believe the major factor contributing to the large burden of S. iniae disease in aquaculture is the lack of fundamental knowledge of S. iniae virulence factors and the fundamental pathogenesis of infection. Our molecular genetic approach to virulence gene discovery and creation of targeted, well-defined attenuated mutants will facilitate rationale design and validation of live-attenuated vaccine candidates. These vaccines offer many theoretical advantages including enhanced

Briefly describe project methodology

Describe progress and accomplishments toward meeting goals and objectives.

We have characterized a number of S. iniae virulence factors using molecular genetic techniques and our newly developed in vitro and established in vivo assays of disease pathogenesis in fish.

In our first year, we discovered the role of the S. iniae phosphoglucomutase (PGM) gene in fish virulence. This gene contributes to cell wall rigidity and capsule expression and loss of PGM leads to an avirulent mutant that is more susceptible to phagocytic and antimicrobial peptide killing. We published this disovery and evidence that shows fish can be immunized with the attenuated PGM-deficient mutant and be protected against subsequent lethal challenge with the WT strain (Buchanan et al. Infection & Immunity 2005.

In the second year, we discovered that the polysaccharide capsule of S. iniae contributes to fish virulence by avoidance of phagocytic clearance and provided preliminary composition analysis on the capsule. The acapsular mutants represent live-attenuated vaccine candidates. These results were published in the new manuscript: Locke JB, Colvin KM, Datta AK, Patel SK, Naidu NN, Neely MN, Nizet V, Buchanan JT. Streptococcus iniae capsule impairs phagocytic clearance and contributes to virulence in fish. J Bacteriol. 2007 189:1279-87.

Also in the second year, we discovered that the beta-hemolysin streptolysin S of S. iniae contributes to fish virulence through a mechanism of direct cytotoxicity to epithelial cells and macrophages. Thus the nonhemolytic mutant represents a live attenuated vaccine candidate. The results are reported in the new manuscript: Locke JB, Colvin KM, Varki N, Vicknair MR, Nizet V, Buchanan JT. The Streptococcus iniae beta-hemolysin streptolysin S is a virulence factor in fish infection. Dis Aquat Organ 2007 (in press). This was also presented as a poster: Locke JB, Colvin KM, Vicknair MR, Varki N, Nizet V, Buchanan JT. Streptolysin S is required for S. iniae infection in fish. 5th International Symposium on Aquatic Animal Health. San Francisco. September 2006.

We have also developed and demonstrated the utility of a variety of in vitro assays in predicting key aspects of the host-pathogen interaction (epithelial adherence and invasion, macrophage phagocytosis and killing, antimicrobial peptide function) in elucidated strain-dependent differences in S. iniae virulence that will help to inform therapeutic and vaccine approaches. These are reported in the submitted manuscript: Buchanan JT, Colvin KM, Vicknair M, Patel SK, Timmer AM, Nizet V. Strain associated virulence patterns of Streptococcus iniae in hybrid-striped bass.

We have discovered a virulence role for four additional S. iniae genes encoding (1) a homologue of GAS M protein, (2) C5a peptidase, (3) a eukaryotic serine/threonine phosphatase regulator; and (4) a polysaccharide deacetylase. These have been demonstrated by precise, allelic replacement mutagenesis and challenges in the hybrid-striped bass infection model. Additional mechanistic studies are well underway and we hope to produce an additional four manuscripts on these topics in the upcoming funding period. This work has been reported in the following poster: Locke JB, Colvin KM, Vicknair MR, Nizet V, Buchanan JT. Characterization of Streptococcus iniae virulence factors for vaccine development.

Aquaculture 2007. San Antonio, TX Feb 2007.

PROJECT MODIFICATIONS: Explain briefly any substantial modifications in research plans, including new directions pursued and ancillary research topics developed. Describe major problems encountered and how they were resolved.

The major goals and research plan has not changed.

We have begun to explore whether zebrafish can represent an alternative model of screening and analysis of S. iniae virulence factors, that would also be amenable to manipulation of aspects of host immunity to help guide rationale for prioritizing vaccine candidates. Collaborations with the experienced zebrafish researchers David Traver (UCSD Biological Sciences) and Lena Gerwick (Scripps Institution of Oceanography) have been established in this regard.

We are testing two new methods for oral delivery of live attenuated S. iniae vaccines. One involves top coating feed with fresh cultured bacteria in combination with a proprietary stomach acid neutralizing preparation developed by researchers at Kent SeaTech or with a novel micrencapsulation technology from researchers at Texas A & M universtiy.

We have gained preliminary access to the complete S. iniae genome contigs from the Baylor University Genome center which has accelerated our basic science and vaccinology goals tremendously.

PROJECT OUTCOMES: Briefly describe data, databases, physical collections, intellectual property, models, instruments, equipment, techniques, etc., developed as a result of this project and how they are being shared.

IMPACTS OF PROJECT: Briefly describe how this project has contributed to a discipline; to developing human resources; to developing physical, institutional or information resources; technology transfer; and society beyond science and technology. Please notify CASG of impacts that occur after your project ends; CASG may contact you after your project ends to learn about additional impacts that occur over time.

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS: Please list any companies, agencies, organizations or individuals who have used your proejct results, scientific/technical advice, etc., and provide names, emails and phone numbers. Briefly describe how results were used and quantify results and socioeconomic benefits, if possible.

The approach to live attenuated vaccination has been protected by the UCSD Technology Transfer office in full partnership with KentSeaTech corporation and we have been in discussion with potential commercial partners including major pharmaceutical companies and larger aquaculture/fish health research organizations.

ECONOMIC BENEFITS generated by discovery, exploration and development of new, sustainable coastal, ocean and aquatic resources (i.e., aquaculture, marine natural products, foods, pharmaceuticals).

Issue-based **forecast capabilities** to predict the impacts of a single ecosystem stressor, developed and used for management (i.e., climate change, extreme natural events, pollution, invasive species, and land resource use).

Tool, technologies and **information services** developed (i.e., land cover data, benthic habitat maps, environmental sensitivity index maps, remove sensing, biosensors, AUVs, genetic markers, technical assistance, educational materials, curricula, training).

Publications (list in appropriate category below) Each listing should be a stand-alone bibliographic reference, including all authors' names. For each Publication type, specify title, authors, date and journal details, where appropriate (repeat headers as necessary).

Conference papers, proceedings, symposia:

Colvin K, Patel S, Timmer A, Van Olst JC, Nizet V, Buchanan J. Strain-associated virulence patterns in Streptococcus iniae in hybrid striped bass. Abstract B223. ASM Conference on Streptococcal Genetics, St. Malo, France, June 2006.

Buchanan JT, Patel S, Datta A, Nizet V. Capsule plays a significant role in virulence in the aquaculture pathogen Streptococcus iniae. Abstract B221. ASM Conference on Streptococcal Genetics, St. Malo, France, June 2006.

Locke JB, Colvin KM, Vicknair MR, Varki N, Nizet V, Buchanan JT. Streptolysin S is required for S. iniae infection in fish. 5th International Symposium on Aquatic Animal Health. San Francisco. September 2006

Locke JB, Colvin KM, Vicknair MR, Nizet V, Buchanan JT. Characterization of Streptococcus iniae virulence factors for vaccine development. Aquaculture 2007. San Antonio, TX Feb 2007.

Peer-reviewed journal articles or book chapters

Buchanan JT, Stannard JA, Lauth X, Ostland VE, Powell HC, Westerman ME, Nizet V. Streptococcus iniae phosphoglucomutase is a virulence factor and target for vaccine development. Infect Immun 2005 73:6935-6944.

Locke JB, Colvin KM, Datta AK, Patel SK, Naidu NN, Neely MN, Nizet V, Buchanan JT. Streptococcus iniae capsule impairs phagocytic clearance and contributes to virulence in fish. J Bacteriol 2007 189:1279-1287.

Locke JB, Colvin KM, Varki N, Vicknair MR, Nizet V, Buchanan JT. The Streptococcus iniae beta-hemolysin streptolysin S is a virulence factor in fish infection. Dis Aquat Organ 2007 (in press).

Buchanan JT, Colvin KM, Vicknair MR, Patel SK, Timmer AM, Nizet V. Strain associated virulence patterns of Streptococcus iniae in hybrid striped bass. (submitted to Veterinary Microbiology)

MEDIA COVERAGE: Select 'Yes' or 'No'. If yes, describe any radio, TV, web site, newspaper, magazine coverage your project has received. Send original clippings or photocopies to the Sea Grant Communications Office.

yes

MEDIA NOTES: Brief description of the type media coverage your project has received.

COOPERATING ORGANIZATIONS: List those (e.g., county or state agencies, etc.) who provided financial, technical or other assistance to your project since its inception. Describe the nature of their cooperation.

INTERNATIONAL IMPLICATIONS: Does your project involve any colleagues overseas or have international implications?

AWARDS: List any special awards or honors that you, or any co-project leaders, have received during the duration of this project.

These are not related to the research alone, but for Victor Nizet:

2006: Elected to the American Society for Clinical Investigation

2006: Elected to the Faculty of 1000 - Biology
2006: Elected to the American Pediatric Society

KEYWORDS: List keywords that will be useful in indexing your project.

Bacterial infection, Streptococcus iniae, aquaculture, fish health, virulence factor, vaccine, hybrid stripied bass,

PATENTS: Please list any patents or patent licenses that have resulted from this project, and complete the patent statement form available on the web site.

yes

NOTES: Please list any additional information in the notes area

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:

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