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SUCCESSFUL FIELD TESTING OF SHRIMP IMMUNOSTIMULANT DEVELOPED BY CIBA

The crustaceans such as the shrimps, lobsters and crabs are a very important group of edible aquatic invertebrates which constitute a major portion of the commercially important seafood products with a global market demand. With the ever-increasing export potential of shrimp, aquaculture of these organisms on a rapid scale brought the problem of diseases. Crustaceans are known to rely on their non-specific immunity to fight diseases. They have an array of non-specific defense mechanisms ranging from presence of a structural hard body covering, to the development of complex enzyme-protein interactions involved in bringing about bacteriostatic or bacteriocidal end-effects. Initiation of these complex defense reactions is triggered by the presence of molecules such as lipopolysaccharides (LPS), peptidoglycans and beta-glucans that constitute the external components of bacteria and fungi.

Immunostimulation is the process of artificial elicitation of immune responses by exposing the shrimp to these recognition molecules or, simply, by exposure to inactivated bacterial/fungal preparations.

Laboratory level investigations

A shrimp immunostimulant (*Vibrio* whole cell-based) was developed, based on several laboratory level experiments carried out under the ICAR AP-CESS fund project entitled "Development and evaluation of shrimp immunostimulants using whole cell preparations of *Vibrio*". Inactivated *Vibrio alginolyticus*, *V. anguillarum*, *V. harveyi*, *V. vulnificus* and *Aeromonas hydrophila*, either individually or in combinations, elicited a significant immune response in tiger shrimp (*Penaeus monodon*) and white shrimp (*Fenneropenaeus indicus*). There was a consistent and significant difference between the immunostimulated and the untreated control groups of shrimp. Protective response and growth were higher in



stimulated shrimp. However, the immune memory (memory factor, MF) which signifies the specificity and long lasting immunity was found to be negligible. Hence, the shrimp required frequent administration of immune elicitors to sustain the immune response. Immunostimulated shrimp also showed enhanced agglutinin, phenoloxidase and bacteriocidal activities.

Preliminary Field Testing-a Success Story

Testing of the immunostimulants under different field conditions was carried out in shrimp farms located at Kattur village (about 50 km north of Chennai), Mahabalipuram (52 km south of Chennai) and Kakdwip Research Centre (KRC) of CIBA, West Bengal. Five trials at Kattur, three trials at Mahabalipuram and two trials at Kadwip were conducted during September 2001 to October 2002 which yielded valuable results. Culture of *P. monodon* was conducted at varying stocking densities (7 to 23 PL/m²) and under different salinity regimes. Healthy seed were procured from commercial hatcheries after confirming that they were PCR



A haul of *P.monodon* (immunostimulated)

negative for white spot syndrome virus (WSSV).

Almost identical method of pond preparation and water management protocols were followed in all these trials. In each farm, a control pond was also maintained



Dr. Mathew Abraham, Director, examining *P. monodon*

The first dose of immunostimulant was given at seventh day of culture (doc) and the subsequent doses were given at weekly intervals. The immunostimulant was mixed thoroughly with feed and fed to the shrimp. Commercial feeds were used in all these farms. All the trials showed significantly better growth and enhanced ability to withstand bacterial necrosis by the immunostimulated shrimp. Field testing of immunostimulant developed by CIBA during *P. monodon* culture has resulted in the production of 1450 kg/ha and 2135 kg/ha in 138 days with four to seven doses of stimulation compared to 1320 kg/ha and 1963 kg/ha obtained from

control ponds. The production levels and disease resistance criteria were taken as indicators to gauge the efficacy of the immunostimulant.

Two immunostimulation trials were also conducted in private shrimp hatcheries.

Immunostimulant was provided via immersion method with nauplius, protozoa, mysis, PL-3 and PL-10 stages of shrimp. All the doses were given via 3 hour-long immersion treatments in the holding tank itself at a very low concentration (105 CFU/ml) of heat-killed *Vibrio* suspension. Control was also maintained. In all these trials, 9 to 12 % higher survival rate of the larvae / postlarvae was recorded.

RESEARCH HIGHLIGHTS

- Bacterial isolates of *Vibrio* species from commercial shrimp hatcheries and farms located around Mahabalipuram near Chennai revealed the presence of the luminescent bacteria such as *V. harveyi* in hatcheries and *V.alginolyticus* in farms.
- Laboratory trials on immune response in Tilapia, *Oreochromis mossambicus* and grey mullet, *Mugil cephalus* were undertaken using rabbit immunoglobulin (IgG). The fish were primed and boosted with rabbit IgG@ 0.4mg/fish with emulsified

Freund's complete adjuvant. Antibody titres from immunized fish estimated by standard ELISA protocols showed good response compared to control fish injected with phosphate buffer.

VISITORS

- Shri Thampi Samraj, Project Director, RGCA, 10 April 2002.
- Dr.T.V.R.S.Sharma, AED, NATP (Coastal Agro-ecosystem); Dr.H.M.Kasim, Officer-In-charge, MRC of CMFRI, Chennai; Dr.K.Nilakantapillai, Professor & Head, Rice Research Station, Tirur; Shri Nallu Chinnappan, Jt. Director of Fisheries (Research), Tamil Nadu State Fisheries Dept., 16 April 2002.
- Dr.A.D.Diwan, ADG (M.Fy.), ICAR, New Delhi, 18 April 2002.
- Dr. K. Gopakumar, DDG (Fy.), ICAR, New Delhi, 19 April 2002.
- Mr.Michael Phillips, Co-ordinator, NACA, Thailand, 31 May 2002 and 20 June 2002.
- Dr.Boonsrim Withyachunnarnkul, Professor, Centex Shrimp, Thailand, Dr.K.Gopal Rao, Professor of Aquaculture, College of Fishery Science, Nellore, Dr.I.Karunasagar, Professor & Head, Dept. of Fishery Microbiology, College of Fisheries, Mangalore; Dr.(Mrs.)

Indrani Karunasagar, Director, UNESCO Centre for Marine Biotechnology, College of Fisheries, Mangalore; Dr.T.Jawahar Abraham, Reader (Microbiology), University of Animal and Fishery Sciences, Kolkata; Prof. R.Madhavi, Vice Chancellor, Sri Padmavathi Mahila Visva Vidyalayam, Tirupathi; Dr.C.V.Mohan, Associate Professor, College of Fisheries, Mangalore; Mr.Pairoj Apiruknusat, Vice President, C.P.Aquaculture (India) Ltd., Chennai; Dr.Peter Walker, Senior Principal Research Scientist, CSIRO Livestock Industries, Australia; Dr.Richard Hodgson, Senior Research Fellow, Centex Shrimp, Thailand; Dr.K.M. Shankar, and Mr.P.C.Thakur, Associate Professors, College of Fisheries, Mangalore; Dr.Tim Flegel, Professor, Centex Shrimp, Thailand; Mrs.A.Uma, Assistant Professor, TANUVAS, Chennai; Mr.B.Vishnu Bhat, Deputy Director, MPEDA, Vijayawada, 20 June 2002.

• Shri Ashok Kumar Tripathy, IAS., Commissioner-cum-Secretary, Fisheries and Animal Husbandry

Department, Govt. of Orissa, 24 June 2002.



Dr.K.Gopakumar, DDG(Fy.), ICAR addressing the scientists at CIBA

ENGAGEMENTS

Dr.Mathew Abraham, Director, attended the following Meetings / Workshops / Seminars:

- Fisheries Divisional Committee Meeting for Zero-Base Budgeting, under the Chairmanship of Dr.K.Gopakumar, Deputy Director General (Fy.), ICAR, New Delhi, during 11-12 April 2002 at ICAR, New Delhi.
- Chaired the NATP-IVLP Site Committee Meeting, under the Institution Village Linkage Programme for Technology Assessment and Refinement in Coastal Agro-ecosystem of Tiruvallur district of Tamil Nadu, on 16 April 2002, at CIBA, Chennai.
- Meeting for Mid-term Review of Vision 2020 in Fisheries Sector, at New Delhi, on 21 May 2002.

केन्द्रीय खारा जलजीव पालन अनुसंधान संस्थान

केखाजपाअसं. द्वारा विकसित झींगा प्रतिरक्षा-उद्दीपक का सफल क्षेत्र परीक्षण

भाकृअनुप ए.पी. सेस की "विब्रियो की संपूर्ण कोशिका के संपाक का उपयोग करते हुए झींगा प्रतिरक्षा उद्दीपकों का विकास तथा मूल्यांकन" नामक परियोजना के अंतर्गत प्रयोगशाला स्तर के कुछ प्रयोगों के आधार पर झींगा प्रतिरक्षा उद्दीपक तैयार किया गया। निष्क्रिय बनाए गए विब्रियो एल्जिनोसैटिकस, वी. एंग्यूल्लारम, वी. हर्वे, वी. वुल्निकफिकस तथा एरोमोनास हाइड्रोफिल्ला (चाहे अलग-अलग या संयुक्त रूप में) टाइगर झींगा (पिनस मोनोडान) तथा सफेद झींगा (फेनेरोपिनस इंडिकस) में महत्वपूर्ण प्रतिरक्षक क्रिया दर्शाते हैं। प्रतिरक्षा उद्दीपक तथा अनुपचारित झींगों के नियंत्रित समूहों में संगत व महत्वपूर्ण अंतर था। उद्दीपक झींगों में रक्षक क्रिया व वृद्धि उच्च थी। जबकि विशिष्टता को प्रदर्शित करनेवाली प्रतिरक्षा स्मृति (स्मृति तत्व, एम.एफ.) तथा लंबे समय तक रहने वाली प्रतिरक्षात्मकता नगण्य दिखाई पड़ी। यद्यपि झींगों में प्रतिरक्षात्मक क्रिया को बनाए रखने हेतु प्रतिरक्षा कारकों के निरंतर उपयोग की आवश्यकता है। प्रतिरक्षा उद्दीपक झींगों में एगग्लुटिनिन, फिनोलॉक्सिडेज तथा बैक्टिरियोसिडल क्रियाएँ भी ज्यादा दिखाई दीं।

सितंबर 2001 से अक्टूबर 2002 के दौरान भिन्न-भिन्न क्षेत्र परिस्थितियों में चेन्नई के महाबलिपुरम् व काट्टूर ग्राम में स्थित झींगा प्रक्षेत्रों तथा केखाजपाअसं के काकट्टीप शोध केन्द्र पश्चिम बंगाल में प्रतिरक्षा उद्दीपकों के परीक्षण किए गए, जिनसे उत्साहवर्धक परिणाम प्राप्त हुए।

पी. मोनोडान के पालन के दौरान केखाजपाअसं द्वारा विकसित प्रतिरक्षा उद्दीपक के क्षेत्र परीक्षण का परिणाम दर्शाता है कि 138 दिनों तक उद्दीपक की चार तथा सात खुराक देने पर उत्पादन क्रमशः 1450 कि.ग्रा./हे. तथा 2135 कि.ग्रा./हे. प्राप्त हुआ जबकि नियंत्रण तालाब द्वारा 1320 कि.ग्रा./हे. तथा 1963 कि.ग्रा./हे. उत्पादन प्राप्त हुआ।

निजी झींगा स्फुटनशालाओं में भी दो प्रतिरक्षा उद्दीपन परीक्षण किए गए। झींगों के नौपलियस, प्रोटोजोइ, माइसिस, पीएल-3 तथा पीएल-10 अवस्थाओं में निमज्जन विधि द्वारा

प्रतिरक्षा उद्दीपक दिए गए। गरमी द्वारा मृत मिश्रित विब्रियो सर्पेशन की बहुत ही कम सान्द्रता वाले (10⁵ CFU/ml) धारण टैंक में सभी खुराकें तीन घंटों के निमज्जन उपचार द्वारा दी गईं। नियंत्रण का भी ध्यान रखा गया। इन सभी परीक्षणों से प्रतिरक्षा उद्दीपक डिम्बक/डिम्बकोत्तर में 9 से 12 प्रतिशत उच्च उत्तरजीविता दर दर्ज की गई।

शोध विशिष्टताएँ:

व्यापारिक झींगा स्फुटनशालाओं व चेन्नई के समीप महाबलीपुरम् के अतराफ स्थित कृषिक्षेत्रों के विब्रियो प्रजाति के जीवाण्विक वियुक्तक, स्फुटनशालाओं में वी. हरवेयी तथा कृषिक्षेत्रों में वी. एल्जिनोलेटिकस जैसे संदीप्तिशील विषाणुओं की उपस्थिति दर्शाते हैं।

टिलापिया, ओरेक्रोमिस मोजंबिकस व ग्रे मुल्लेट, मुगिल सेफालस में प्रतिरक्षा के लिए खरगोश इम्यूनोग्लोब्यूलिन (IgG) का उपयोग करते हुए प्रयोगशाला में परीक्षण किये गए। खरगोश (IgG) (0.4 मि.ग्रा./मछली की दर से) के साथ पायसीकृत फ्रेउंडस पूर्ण सहोषद देने से मछलियों की गुणवत्ता उत्तम रही व उनका उत्पादन बढ़ा। फास्फेट बफर से इंजेक्ट की गई मछलियों की अपेक्षा मानक एलिसा प्रोटोकाल द्वारा आकलित रोगप्रतिकारक टिट्रेस द्वारा प्रतिरक्षित मछलियों में प्रतिक्रिया अच्छी दिखाई पड़ी।

बैठकें:

डॉ. मात्यू एब्रहाम, निदेशक केखाजपाअसं. की अध्यक्षता में 16 अप्रैल, 2002 को चेन्नई में राकृप्रौप - संगोंसंका (जैसे तमिलनाडु के तिरुवल्तूर जिले के तटीय कृषि-परितंत्र में तकनीकी निर्धारण व परिशोधनार्थ संस्थान व गांव का संयुक्त कार्यक्रम) की साईट समिति की बैठक हुई।

27 अप्रैल, 2002 को केखाजपाअसं. चेन्नई में संस्थान की वार्षिक कर्मचारी शोध परिषद् की बैठक हुई। उक्त बैठक में डॉ. ए. दिवान, उप-महानिदेशक (स.मा.) भाकृअनुप, नई दिल्ली उपस्थित थे।

7 जून 2002 को केखाजपाअसं. मुख्यालय चेन्नई में संस्थान प्रबंध समिति की 23वीं बैठक हुई।

केखाजपाअसं. - एन.ए.सी.ए. विशेषज्ञों की परामर्श बैठक

संस्थान ने अपने मुख्यालय चेन्नई में 12-14 जून तक नेटवर्क आफ एक्वाकल्चर सेन्टर्स इन एशिया-पेसेफिक्स (एन.ए.सी.ए.) थाईलैण्ड के साथ झींगों में विषाण्विक रोगों का शीघ्र पता लगाने हेतु तीन दिन तक विशेषज्ञ परामर्श बैठक का आयोजन किया। बैठक में जलकृषि अनुसंधान से संबंधित विभिन्न क्षेत्रों के 41 विशेषज्ञ उपस्थित थे जिनमें 5 बाहर के तथा प्रगतिशील कृषक भी सम्मिलित हैं। इस कार्यशाला में पी.सी.आर. प्रोटोकाल के मानकीकरण की विधि पर चर्चा की गई।

मानव संसाधन विकास

संस्थान के वैज्ञानिकों ने "कृषि अनुसंधान परियोजना प्रबंधन में अत्याधुनिकता" तथा "एडवांस साफ्टवेयर" पर आयोजित प्रशिक्षण कार्यक्रमों में भाग लिया।

प्रशिक्षण

संस्थान द्वारा 17-22 जून, 2002 तक "केकड़ा प्रजनन व पालन" पर प्रशिक्षण कार्यक्रम आयोजित किया गया।

केखाजपाअसं. मुख्यालय व मुत्तुकाडु प्रायोगिक केन्द्र पर व्याख्यान तथा प्रदर्शन आयोजित किए गए।

एक्वा फेयर में सहभागिता

संस्थान ने 27-28 अप्रैल, 2002 को केन्द्रीय अंतरस्थलीय प्रग्रहण मात्सियकी अनुसंधान संस्थान, बैरकपूर में आयोजित "अंतरस्थलीय जल में मात्सियकी को बढ़ावा - चुनौतियों का सामना" पर राष्ट्रीय परिसंवाद से संबंधित एक्वा फेयर में भाग लिया।

स्टाफ समाचार

नियुक्ति

26 अप्रैल, 2002 को श्री एस. राजमाणिकम् ने टी-4 के रूप में कार्यभार ग्रहण किया।

प्रत्यावर्तन

श्री के.वी. डेल्ली राव को उनके निवेदन पर 6 अप्रैल, 2002 से पुनः एस.एस.जी.आर. I पद पर बहाल किया गया।

The scientific, technical and administrative personnel attended the following meetings / seminars / workshops:

- Dr.B.P.Gupta, Principal Scientist and Mrs. P.Mahalakshmi, Scientist, participated in the National Conference on Coastal Agricultural Research, during 6-7 April 2002, at ICAR Research Complex, Goa.
- Shri K.Ponnusamy, Scientist, visited Tamilnadu Agricultural University, Coimbatore, from 22-26 April 2002, to discuss about the technological interventions to be carried out during the year 2002-2003 under the NATP-IVLP Project viz., Institution Village Linkage Programme for Technology Assessment and Refinement in coastal Agro-ecosystem of Tiruvallur district of Tamil Nadu.
- Dr.B.P.Gupta, Principal Scientist, participated in the Symposium on Seafood Safety - Status and Strategy, held at CIFT, Cochin, during 28-30 May, 2002.
- Dr.B.P.Gupta, Principal Scientist, attended the Meeting on Network Project entitled "National Risks Assessment Programme for Fish and Fish Products for Domestic and National Markets, held at CIFT, Cochin, on 30 May 2002.
- Dr.(Mrs.) M.Jayanthi and Dr.M.Muralidhar, Scientists (Senior Scale), attended the training programme on

"ERDAS IMAGINE 8.5", organized by M/s ESRI India Ltd., at Chennai, during 20-24 May 2002.

- Dr.P.Ravichandran and Dr.N.Kalaimani, Principal Scientists, Dr.K.K.Vijayan, Senior Scientist and Shri S.V.Alavandi, Scientist (SG), participated in the National Seminar on "Shrimp Diseases Management - Selection of Best Species for Shrimp Culture and Health Management", held at Nellore, Andhra Pradesh, on 15 June 2002.
- Shri K.Ponnusamy, Scientist, participated in the Annual Review Workshop under NATP Agro-ecosystem organized at CTCRI, Trivandrum, during 20-21 June 2002 and presented the annual plan of the NATP-IVLP project for Technology Assessment and Refinement in coastal Agro-ecosystem of Tiruvallur district of Tamil Nadu .

MEETINGS

NATP-IVLPMEEETING

The Site Committee meeting of the NATP - IVLP programme viz., "Institution Village Linkage Programme for Technology Assessment and Refinement in Coastal agro-ecosystem of Tiruvallur District of Tamil Nadu" was held on 16 April

2002, at Chennai, under the Chairmanship of Dr.Mathew Abraham, Director, CIBA. Dr.T.V.R.S.Sharma, Director, Coastal Agro-ecosystem, Pedavegi; Dr.K.Nilakantapillai, Professor & Head, Rice Research Station, Tirur; Dr.H.M.Kasim, Officer-in-Charge, MRC of CMFRI, Chennai; Shri Nallu Chinnappan, Joint Director of Fisheries (Research), Tamil Nadu State Fisheries Dept., Chennai and Scientists from CIBA participated in the meeting.



NATP-IVLP Site Committee Meeting at CIBA

STAFF RESEARCH COUNCIL MEETING

Annual Staff Research Council Meeting of the Institute was held at CIBA, Chennai, on 27 April 2002.



Dr. A. D. Diwan, ADG(M.Fy.), ICAR interacting with scientists at the SRC meeting
Dr.A.D.Diwan, Assistant Director

General (M.Fy.), ICAR, New Delhi, attended the meeting.

INSTITUTE MANAGEMENT COMMITTEE MEETING

The 23rd Meeting of the Institute Management Committee was held at CIBA Headquarters, Chennai, on 7 June 2002.

CIBA - NACA EXPERT CONSULTATION MEETING

The Institute along with Network of Aquaculture Centres in Asia-Pacific (NACA), Thailand, jointly organized a three day Expert Consultation Meeting on Rapid Diagnosis of Shrimp Viral Diseases, at CIBA Headquarters, Chennai, during 12-14 June, 2002. 41 experts from various fields of research in aquaculture including five from abroad and progressive farmers attended the meeting. The workshop discussed the methods to standardize the PCR protocol followed by various researchers in order to get consistent results for rapid and accurate diagnosis of white spot virus disease in shrimps. The PCR techniques followed in India were critically examined by the



CIBA-NACA expert consultation meeting at Chennai

expert group and the need for collaborative research and knowledge-sharing in the area of shrimp health management was highlighted.

HUMAN RESOURCE DEVELOPMENT

- Dr.C.P.Balasubramaniam, Scientist (SS) and Dr.(Mrs.) B.Shanthi, Scientist, participated in the training programme on “Recent Advances in Agricultural Research Project Management”, at NAARM, Hyderabad, during 15 April to 5 May 2002.
- Mrs.P.Mahalakshmi, Scientist, attended the training programme on “Advanced Software Training Covering RDBMS Concepts, MS Access, Networking & Oracle Essentials, D2K, GIS & Spatial Data Management Concepts” from April 15 to May 16, 2002 at NIIT, New Delhi.

TRAINING

- A training programme on “Crab breeding and culture” was organized by the Institute from 17-22 June 2002 at CIBA, Chennai. Two research scholars from Centre of Advanced Studies in Marine Biology, Annamalai University, Parangipettai and a private fish hatchery operator from Chennai, participated.

Lectures and demonstrations were conducted for the following at CIBA, Chennai and Muttukadu Experimental Station:

- 5 final year B.F.Sc. students from College of Fisheries, G.B.Pant University, U.P., during 2-3 May 2002.
- 12 trainees from Staff Training Institute, Dept. of Fisheries, Govt. of Tamil Nadu, on 13 May 2002.
- 5 students from Central Agricultural University (IMPHAL), College of Fisheries, Tripura, on 17 May 2002.

PARTICIPATION IN AQUA-FAIR

CIBA participated in the Aqua-Fair conducted in connection with the National Symposium on “Fisheries Enhancement in Inland Waters-Challenges Ahead”, held at Central Inland Capture Fisheries Research Institute, Barrackpore, Kolkata, during 27-28 April 2002.

STAFF NEWS

Appointment

- Shri S.Rajamanickam joined as T-4, on 26 April 2002.

Reversion

- Shri K.V.Delli Rao, Junior Clerk, on his request was reverted to the post of Supporting Staff Gr.I, with effect from 6 April 2002.

