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Report on a visit to CIFT, Cochin,
India to provide training and
guidance in studying
microbiological problems in
cultured fish/seafoods - 13
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Abbreviations

API	Analytical Profile Index
CIFT	Central Institute of Fishery Technology
EIA	Export Inspection Agency
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
HPLC	High Performance Liquid Chromatography
MFB Section	Microbiology, Fermentation and Biotechnology Section
NRI	Natural Resources Institute
ODA	Overseas Development Administration

Summary and Recommendations

1. A visit was made to the Central Institute of Fisheries Technology (CIFT), Cochin as part of the ODA funded collaborative programme between NRI and CIFT. The aim of the work was to further strengthen the skills of CIFT staff and to help in institution building. Staff within the Microbiology, Fermentation and Biotechnology Section (MFB Section) were given training in the identification of selected microbial pathogens and guidance in undertaking research studies at CIFT into the microbiological problems likely to be encountered with the spread of aquaculture systems in India.
2. A number of recommendations arose from the visit. These are detailed below.
3. It is **recommended** that staff in the MFB Section adopt a more team-oriented approach to project work, with regular project meetings to discuss results and plan future work.
4. It is **recommended** that staff are encouraged to make more use of the computer facilities at CIFT.
5. It is **recommended** that the results obtained using rapid test kits should be confirmed using traditional microbiological techniques incorporating suitable positive and negative controls.
6. As input to the CIFT programme of assistance from ODA it is **recommended** that a four week visit, by a microbiologist, should be made to India, to train CIFT microbiologists (Quality Control Laboratory, MFB Section and outposts of CIFT) in the isolation and identification of *Vibrio vulnificus* and the use of traditional and minituarised test kits (API system, marketed by bioMerieux of France) for the routine confirmation of the identity of a range of food pathogens, including *Salmonella* spp., marine vibrios, *Vibrio cholerae*, *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Listeria*

monocytogenes. A request from Dr Iyer of the Quality Control Laboratory has been made for these activities.

7. It is **recommended** that API test kits for a range of pathogens should also be introduced, by CIFT staff (after training), to microbiologists at quality control laboratories in local seafood industries.

8. The Quality Control Laboratory at CIFT performs analyses for the Export Inspection Agency of India. If the EIA obtains European Union (EU) competent authority status, the Quality Control Laboratory would be classed, by the EU, as an official control laboratory subject to the rules and regulations of Council Directive 93/99/EEC (additional measures concerning the official control of foodstuffs). The laboratory would have to comply with the general criteria for the operation of testing laboratories laid down in European Standard EN 45001 supplemented by standard operating procedures and the random audit of their compliance by quality assurance personnel. These regulations come into force from the 1 November 1998. If these conditions are not met the laboratory will not be recognised by the EU.

9. In view of the conditions being laid down by the EU regarding exports from third countries to the single market, it is **recommended** that a review be made of the microbiological procedures currently being used at the various outstations of CIFT to ensure that all the microbiology staff at CIFT are using the same recommended techniques (appropriate to local conditions) and following good laboratory practice. As input to the CIFT programme of assistance from ODA it is **recommended** that a microbiologist should undertake a two to three week visit to India to carry out this review. The microbiologist would also provide advice to CIFT on improvements required to existing microbiology facilities at the various outstations and the procedures which need to be adopted by CIFT to fulfil the requirements to the EU Council Directive 93/99/EEC.

10. It is **recommended** that laboratory safety is improved within the microbiology laboratories. Mouth-pipetting should be stopped immediately and staff should be stopped from eating or drinking within the laboratory. Staff should wear protective clothing whilst working in the microbiology laboratories; this may only become practicable when new air-conditioned laboratories have been commissioned.

Introduction

11. CIFT undertakes research investigations in all disciplines relating to fishing and fish processing. The Institute has close links with seafood industries at all levels. Staff provide advice on handling procedures, quality standards and quality assurance to exporters of seafoods and advise the Government of India, State Fisheries Departments and local fisherfolk on the reduction of post-capture losses in fisheries.

12. Microbiological activities at CIFT are undertaken within the Quality Control Laboratory of the Fish Processing Division and within the MFB Section. The Quality Control Laboratory is responsible for the routine analysis of samples of fish and shrimp sent to the Institute by seafood exporters and undertakes some applied research into microbiological problems associated with seafoods. However, the majority of the microbiological research activities are performed within the MFB Section.

13. A visit was made by a microbiologist from NRI to CIFT to strengthen the skills of CIFT staff in the identification of microbial pathogens and to guide research studies at CIFT into the microbiological problems likely to be encountered with the spread of aquaculture systems in India. Terms of reference for the visit are detailed in Appendix 1.

Background

14. The Quality Control Laboratory of the Fish Processing Division is responsible on a contract basis for the routine analysis of samples of seafoods destined for export. Results of the analysis are conveyed to the Export Inspection Agency (EIA), who subsequently issue health certificates. The laboratory also undertakes microbiological and chemical research based on the requirements of the local seafood industry. Recent research has included studies of the

survival of *Listeria monocytogenes* in shrimp during freezing and frozen storage, the mycoflora of dried fish obtained from Cochin fish markets and the incidence and fate of human pathogens in cultured *Penaeus monodon*.

15. The MFB Section is currently undertaking investigations into toxigenic and pathogenic bacteria associated with marine and cultured fishes. A project entitled "Investigations of aquatic microorganisms with special reference to pollution, fish preservation, pathogenesis and bioactivity" has been started. The main objectives of the project are to study: the incidence of pathogenic bacteria in aquatic environments; microbial pollution of aquatic and aquaculture systems; the fate of human pathogens during fish processing; the development of new fish preservation methods using lactic acid fermentation and; the screening of microorganisms isolated from fish and the aquatic environment for the production of bioactive and antibiotic substances and development of antibiotic resistance. So far the project has involved a study of the incidence of *Listeria* in prawns and marine fish, an investigation of the cause of epizootic ulcerative syndrome in fish in Kerala and the incidence of pathogenic bacteria and antibiotic resistant bacteria in commercial fish products.

Training activities at CIFT

16. Training activities undertaken at CIFT are summarised in Appendices 2 and 3. The following observations and recommendations arose from the training programme.

Project design

17. Background information and the progress of the project entitled "Investigations of aquatic microorganisms with special reference to pollution, fish preservation, pathogenesis and bioactivity" were discussed with the head of the MFB Section Dr Surendran, and three of the other scientists/senior scientists working on the project.

Suggestions for future work were also discussed. It became evident during these discussions that there was a weakness in the ability of staff to identify new areas of research and implement projects. Members of the section were shown the importance of taking programmes through a complete project cycle including identifying the need for the research and the beneficiaries, project design, costing of inputs, monitoring the progress and evaluating the outcomes of the project. The use of logframes and concept notes during project formulation was also discussed.

18. There was also a noticeable lack of project reports making it difficult to determine the extent of progress. The need for regular scientific progress reports and project planning meetings was emphasised. Currently research findings are often confined to individual laboratory notebooks and are only made available to a wider audience at the time of publication in a scientific journal. It is **recommended** that a more team-oriented approach to project work is adopted. Training in the use of spreadsheets/computers for the recording of results and the preparation of reports and scientific publications was given to Dr Sanjeev of the Quality Control Section and members of the MFB Section. None of these staff had any previous experience of using a computer. The MFB Section intends to purchase a computer in the near future. It is **recommended** that staff are encouraged to use the computer facilities at CIFT, initially for the recording and analysis of results and the preparation of project reports and scientific publications. Laboratory based staff at CIFT should be provided with training in basic computer skills.

Review of existing microbiological methods

19. Current methods for the microbiological examination of fish and shellfish used in the MFB Section were reviewed and modifications, where appropriate, suggested. At present the section has the capability to analyse food samples for a variety of pathogenic bacteria including *Salmonella*, *Vibrio*

parahaemolyticus, *Vibrio cholerae*, *Clostridia*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Bacillus cereus*, coliforms and *Listeria*. Rapid methods have been adopted for many of the analyses with a heavy reliance on imported immunochemicals and kits.

20. Microbiological media and biochemicals are available in India, however, the laboratory staff at CIFT have expressed doubts about the consistent quality of those produced and purchased locally. Procurement by CIFT of microbiological media, immunochemicals, rapid kits and biochemicals has to be planned well in advance as up to one year may elapse between the time of placing an order and the receipt of the goods. Many of the immunochemicals and kits purchased have limited shelf-lives and require constant refrigeration. As a result, at the time of receipt at CIFT, many of these have already passed their expiry date or been subjected to temperature abuse. The hazards of using expired reagents and kits were emphasised to the laboratory staff and demonstrated in the laboratory. If rapid test kits are to be routinely used, it is **recommended** that the results should be confirmed using traditional microbiological techniques with suitable positive and negative controls.

21. It is important that the microbiological methods recommended to CIFT during training courses are applied across all the microbiology laboratories. Evidence obtained during the visit to CIFT suggested that even adjacent laboratories at CIFT, Cochin are following different methods for the same organism.

22. Two field visits were made with CIFT staff to shrimp farms in Cochin and Mandapan (Tamil Nadu) to evaluate the methods being used by the MFB Section and the Quality Control Laboratory for the taking of samples of shrimp, sediment and water samples for microbiological examination. The methods used in each case were found to be scientifically valid. The importance of statistically valid sampling and the unbiased

selection of sites for study was explained to the staff involved.

New microbiological techniques

23. A number of newly emerging pathogens associated with seafoods have become of importance to the food microbiologist. Recently interest has increased in human pathogens such as *Listeria monocytogenes*, *Aeromonas hydrophila*, *Escherichia coli* 0157 and *Vibrio vulnificus*. Countries importing seafoods from India require high standards of microbiological quality. Zero tolerance levels for organisms such as *Listeria monocytogenes*, *Vibrio cholerae* and *Salmonella* have been set by many importers. Attention is now being focused on some of the other serious food pathogens such as *Vibrio vulnificus* and *Escherichia coli* 0157.

24. To increase the technical expertise of CIFT, staff of the MFB Section received brief training in the analysis of *Escherichia coli* serotype 0157 using a fluorescent agent 4-methylumbelliferyl-B-D-glucuronide incorporated into traditional microbiological media along with the use of MacConkey Sorbitol agar. The final confirmation stage of this analysis requires the use of a latex agglutination kit (Unipath, UK), again with a limited shelf life. A request was received from Dr Iyer of the Quality Control Laboratory for training of staff in the identification of *Vibrio vulnificus* from seafoods. The organism is a serious food pathogen and a zero tolerance level for this organism in seafoods may be set in the future. It is **recommended** that microbiologists at CIFT are trained in the identification and isolation of *Vibrio vulnificus* from seafoods using a combination of traditional techniques and API (bioMerieux) standardised and miniaturised test system.

26. The use of antimicrobials in aquaculture at both therapeutic and sub therapeutic levels has become widespread. The possible development of antibiotic resistance in human

pathogens resulting from the use of medicated feeds is a cause for concern. To enable CIFT to undertake studies of the development of antibiotic-resistant pathogens in aquaculture systems training was given in the determination of antibiotic-resistant organisms using the standardised single disk antibiotic susceptibility testing method of Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck M. (1965). CIFT were provided with a new antibiotic disc dispenser and a supply of antibiotic discs to enable this work to continue. Methods for the determination of antibiotic residues in seafoods were discussed with Mr Anthony of the Biochemistry and Nutrition Division. Standard Operating Procedures (SOP's) for the High Performance Liquid Chromatographic (HPLC) determination of chloramphenicol, sulphonamides and tetracyclines in animal tissues were left at CIFT. A number of consumable items such as HPLC columns and clean-up cartridges would need to be purchased by CIFT before these methods could be implemented.

Constraints to using microbiological methods

27. In the microbiology laboratories some difficulties were evident in performing many of the routine biochemical tests required for the positive identification of food pathogens. The sole use of immunological test kits for confirmation of foodborne pathogens cannot be totally relied upon. It is **recommended** that microbiologists at CIFT should be trained in the use of standardised and miniaturised tests such as those marketed by bioMerieux of France as "API strips" for during routine identification of organisms such as *Salmonella*, *Vibrio* and *Aeromonas* spp, *Plesiomonas shigelloides* and *Listeria* spp. These rely on performing a number of traditional microbiological tests in a series of microtubes. Results are relatively reliable and the methods are internationally recognised. A computer database is available to simplify the interpretation of results.

28. Problems have been encountered at CIFT with obtaining sheep's blood for the final differentiation of *Listeria*

monocytogenes from non-pathogenic *Listeria* spp. by the CAMP test. An alternative method would be the use of the API *Listeria* (bioMerieux) standardised and miniaturised test system which permits the characterisation of the genus *Listeria* without the need of a CAMP test. It is **recommended** that API test kits for a range of pathogens should also be introduced to microbiologists at quality control laboratories in local seafood industries.

Laboratory safety

28. During the training activities at CIFT a number of bad practices were observed within the Microbiology laboratory, including mouth-pipetting of hazardous chemicals and microbiological cultures, eating and preparation of food, drinking of water and the lack of protective clothing, particularly where staff are wearing highly flammable items such as silk saris. The benches, equipment and chemicals within the laboratory were also observed to be covered in a light film of dust. The build up of dust is due to the lack of air-conditioning within the laboratory and the consequent need for windows to be left open and the use of electric fans.

29. It is **recommended** that mouth-pipetting should be stopped immediately and staff should be stopped from eating or drinking within the laboratory. New, more spacious, microbiology laboratory facilities at CIFT are planned for the near future. These will be air-conditioned and therefore more readily kept clean. When these facilities have been completed, it is **recommended** that staff wear protective clothing whilst working in the microbiology laboratory. These basic measures will give the laboratory a more 'professional' appearance which will become more important with the development of closer relationships with the seafood industry.

Appendix 1 Terms of Reference

The terms of reference for the visit were:

- a) To guide studies into the survival, growth and spread of human pathogenic bacteria (with particular reference to newly emerging pathogens) in selected aquaculture systems in India.
- b) To enable CIFT to determine the incidence of antibiotic resistant human pathogens in aquaculture systems and the surrounding environment in India.
- c) To train CIFT staff in methods for the identification and isolation of newly emerging human pathogens.
- d) To report on the findings within one month of returning to the UK.

Appendix 2 Training of staff within the Microbiology Section at CIFT

The following staff within the MFB Section received training during the visit:

Dr P. K. Surendran
Dr Nirmala Thampuran
Dr S. Sanjeev
Shri V. Narayanan Nambiar

Dr Sanjeev transferred from the MFB Section to the Quality Control Laboratory during the period of training.

The following training was provided to these staff:

- i) Review of existing microbiological methods for identification and enumeration of foodborne pathogens and modification of procedures where appropriate. Review of sampling procedures and sample site selection for study of survival and spread of human pathogenic bacteria in aquaculture systems.
- ii) Training in the isolation/enumeration of *Escherichia coli* 0157 from seafoods.
- iii) Training in the determination of antibiotic-resistant microorganisms using the standardised single disk antibiotic susceptibility testing method.
- iv) Brief training in the use of log frames and project cycles, including identifying the need, designing the project, costing the inputs, undertaking the research, monitoring the progress and evaluating the outcomes.
- v) The use of computers/spreadsheets for storing and reporting results. Introduction to the preparation of

scientific publications using word processors and graphics-based computer software.

Appendix 3 Itinerary of the visit

Monday 13 February

Review of existing microbiological methods used in the MFB Section. Meeting with Dr Surendran, Head of MFB Section.

Tuesday 14 February

Laboratory methods, report writing and project formulation.

Wednesday 15 February

Field trip to fish farm in Cochin area to review and recommend methods for microbiological sampling from aquaculture systems.

Thursday 16 February

Basic computer familiarisation. Use of word processors and spreadsheets for producing laboratory reports, storage of results and writing of scientific publications.

Friday 17 February

Discussion of laboratory methods for the isolation and enumeration of *V. parahaemolyticus* and *V. cholerae*.

Monday 20- Tuesday 21 February

Project cycles including project identification, project monitoring and the use of logframes.

Wednesday 22 February

Field trip to Mandapan, Tamil Nadu. All day travelling.

Thursday 23 February

Field trip to Mandapan. Visit to two shrimp farms. Sampling of shrimp, pond water and sediment for subsequent analysis in the Quality Control laboratory.

Friday 24 February

Return to Cochin. All day travelling.

Monday 27 February

Public Holiday. Working in Hotel.

Tuesday 28 February

Discussion of the field visit to Mandapan. Start of analysis of samples taken in Mandapan for human pathogens. Continuation of computer training.

Wednesday 1 - Friday 3 March

Visit to the College of Fisheries, Mangalore to review progress of EMC X0238 with project leader Dr Karunasagar. (not part of the CIFT activities).

Saturday 4, Monday 6 - Wednesday 8 March

Training in the detection of the human pathogen *Escherichia coli* 0157 and methods for the detection of antibiotic resistant pathogens. Discussion of the use of latex test kits and demonstration of problems with using out of date reagent kits. ELISA and Oxoid rapid *Salmonella* test methods.

Thursday 9 March

Summing up of training provided. Discussion of future training needs with Dr Iyer, Head of Quality Control laboratory. Meeting with Dr Gopakumar, Director of CIFT.

Friday 10 March

Departure for UK.