

**RESEARCH REPORT FOR DARWIN
PROJECT:**

**BACTERIAL DIVERSITY IN COASTAL
SEAWATER IN SHANDONG PROVINCE,
CHINA**

REFERENCE: 162/08/170

**PARTNERS:
OCEAN UNIVERSITY OF QINGDAO
HERIOT-WATT UNIVERSITY**

March, 2002

DARWIN INITIATIVE

FINAL REPORT

1. Basic Project Details

Project Title: **Bacterial diversity in coastal seawater in Shandong Province, China**

Contractor: Heriot-Watt University, Edinburgh

Host country collaborating institute: Peoples Republic of China; Ocean University of
Qingdao

Grant Round: 1999/2000; Round 5

Grant Value: £120,938

2. Project Expenditure

Total grant expenditure: £120,938

Breakdown of expenditure (using expenditure categories in the original application
form)

Staff:

Travel:

Printing:

Conference:

Capital items:

Other:

Actual spend:

Staff:

Travel:

Printing:

Conference:

Capital items:

Other:

The reasons for variations of +/- 10% are, as follows:

Travel: this category included travel and accommodation costs associated with the Conference (= Workshop).

Conference: the apparent underspend was that some of the travel and accommodation costs were included under the heading of travel.

Capital items: the increased cost reflected that the Sub-contractor purchased some small items, the cost of which exceeded Heriot-Watt's threshold, and have therefore been included in this category.

3. **Project Background/Rationale**

Why was the project needed? Please explain the project development process.

The results of an EU-STD3 project between Belgium, China, Ecuador and the UK indicated that pronounced effect of industry and aquaculture on the composition of the coastal marine bacterial communities, especially in developing countries. However, the previous work highlighted shrimp-pathogenic vibrios, and largely ignored the other bacterial representatives of the coastal environment at the experimental stations in Shandong Province, China. The possible impact in changes of the bacterial communities on human and animal health was noted. Also, the effect on marine biotechnology was unquantifiable. The previous study established a working relationship with scientists at the Ocean University of Qingdao (OUQ), who recognised that a natural, balanced bacterial community is essential to maintain the wellbeing of the coastal environment as well as providing an invaluable resource for biotechnology. China is well aware of potential problems regarding the outcome of an imbalance in microbial communities. By means of Email, telefax and letters, the interest of OUQ was awakened, and Professors Xu and Austin developed the proposal. After the funding was awarded, frequent meetings and use of Email, telefax and letter allowed the project to develop. Thus, research and training needs were identified, and the relevant programmes developed.

How was it related to conservation priorities in the host country?

The perceived need to maintain a natural, balanced bacterial community in the coastal marine environment, and to train (local) staff in the appropriate methodologies to study microbial biodiversity.

How was the project intended to assist the host country to meet its obligations under the Biodiversity Convention?

This Darwin Initiative project was designed to assist in furthering the fulfilment of China's obligations under several articles of the Biodiversity Convention, mainly in assessment and monitoring, training and public awareness/environmental awareness.

Was there a clear 'end-user' for the project in the host country? Who?

The end user was OUQ, who reported to the relevant ministries in Beijing and Shandong Province.

4. Project Objectives

Æ What were the objectives of the project (as stated in the original application form)?

- To establish a unit within the Ocean University of Qingdao (OUQ) to specialise in the development of methods for the study of bacterial biodiversity in coastal seawater habitats
- To initiate a programme of data collection of biological, physical and chemical parameters at polluted sites in Shandong Province. Polluted sites will be in the vicinity of industrial and aquacultural discharges. Clean sites, which have been already identified, are in rural areas without any industrial discharges. Samples will be taken for bacteriological examination using agreed methods.
- To train Chinese scientists during training courses in the UK and in Qingdao on the techniques and methodology relevant for bacterial isolation and identification.
- To assist OUQ in promoting its activities of increasing awareness of fragility of Shandong's coastal habitats and the need for sustainable management of them among local residents, industry and visitors.
- To assist OUQ, once project specific local expertise and reputation is established, in attracting further research and development funding for the extension of microbiological studies in the coastal waters of Shandong.
- To extend and develop Heriot-Watt University's staff and research student expertise in marine microbiological problems in China.

Æ Were the objectives of the project revised? If so, how?

No

Æ Have the objectives (or revised objectives) been achieved? If so, how?

- The Darwin Unit was established in OUQ (a photograph of the official plaque is enclosed). The team of microbiologists developed expertise to study coastal marine bacterial biodiversity.
- Detailed discussions in Edinburgh and Qingdao led to the development of a realistic research programme. Specifically, suitable sites were identified, and a programme of sample collection devised. Appropriate microbiological, and physico-chemical methods were refined/developed. A detailed report from OUQ is appended..
- Chinese scientists were trained in environmental microbiology and bacterial identification methods during two courses in Edinburgh and in follow-up visits to Qingdao. These researchers were then instrumental in training Chinese technician and undergraduate and postgraduate students.
- Apart from the activities of Professor Xu in highlighting concerns to national and provincial ministries, the Workshop provided an ideal opportunity of promoting the problems to Chinese scientists and the hierarchy of the OUQ.
- The award of this Darwin initiative project, heightened the international prestige of OUQ in the eyes of the Chinese government. This led to the award of 900 million Yuan to enhance the research and teaching base of the University. Also, UNESCO awarded Professor Xu an unspecified sum to train scientists from developing countries in microbial biodiversity.
- Certainly, Edinburgh-based staff profited from the collaboration, enhancing the knowledge base and expertise. Professor Austin was awarded the title of Guest Professor of the Ocean University of Qingdao.

To extend and develop Heriot-Watt University s staff and research student expertise in marine microbiological problems in China.

Æ If relevant, what objectives have not been achieved, or only partially achieved, and why?

All objectives have been achieved.

5. **Project Outputs (see the attached list of project outputs which we would like you to use in compiling this section of the report)**

Æ What output targets, if any, were specified for the project? (Please refer to the project schedule agreed with the Department where relevant.)

1999/2000

6A/B 2 OUQ staff receive 3-weeks training in the UK in isolation and identification of bacteria

8 2 UK staff visit Qingdao to discuss progress and further work

6A/B At least 2 Chinese scientists receive training for at least 12-weeks

2000/2001

6A/B 2 Chinese scientists are trained in rapid identification techniques in the UK for 3-weeks

2001/2002

8 2 UK scientists visit OUQ to discuss and take part in the analyses

14A Workshop held for at least 10 leading Chinese scientists from other institutions

11B 4 (scientific) papers produced

12A Database produced and handed over to OUQ; Final report produced for Darwin

Æ Have these been achieved?

Yes

Æ If relevant, what outputs were not achieved, or only partially achieved, and why?

All outputs were achieved.

Æ Were any additional outputs achieved?

1B Associate Professor Xiao-Hua Zhang was registered as a Ph.D. student at Heriot-Watt University, and was recently awarded the degree of Doctor of

Philosophy. Subsequently, she was awarded a postdoctoral research position for 3-years in molecular biology at the University of Oxford.

- 20 Largely on the basis of this contract, i.e. international recognition, OUQ received 900 million Yuan to further their research and training activities in marine science.

In addition, the OUQ had technicians, undergraduate and postgraduate students working on the project. Two Ph.D. students at Heriot-Watt University were engaged on related work, comparing the microbiology of the Chinese sites with comparative areas in Scotland. Both students are due to complete the work in autumn, 2002.

- Æ If output targets were not specified, please state the outputs achieved by the project. As far as possible, we would like you to work through the list of outputs attached to this paper and to report on those, which are relevant to your project.

As above

6. Project Operation/Management

- Æ Research projects - please provide a **full** account of the scientific work undertaken, outlining the methodology adopted, the staff employed and the research findings. The extent to which research findings have been subject to peer review should be addressed.

A detailed research report, as produced by OUQ, is appended. Similarly, the account of the research activities in Edinburgh is provided at the end of this report. Briefly:

Methodology: The project involved determination of the physico-chemical parameters of the water and sediment samples using established methods, and the use of spread plating techniques to isolate bacteria. Random collections of bacterial cultures were characterised by means of BIOLOG-GN (with numerical analyses) and cell protein profiling.

Staff employed: Heriot-Watt University engaged the part time services of a research Associate (Dr. P.A.W. Robertson). Two Ph.D. students (Libby Jordan and James MacInnes were involved with the project). OUQ employed three full-time researchers (Zong-Jun Du, De-Hua Yu and Xiang-Hong Wang), and a part-time postdoctoral fellow (Ji-Xiang Chen). Also, the following postgraduate students were engaged on the work: Wan-Li Gou, Shuang Li, Ju Sheng, Bao-Kun Wang, Peng Wang, Yong Yu and Gui-Li Zheng.

Research findings: These are indicated in the appendices.

Peer review: Articles are already in press with the Journal of the Ocean University of Qingdao (peer-reviewed) and Annales of the New York Academy of Science (peer reviewed):

1. Zong-Jun Du¹, Yun Li¹, De-Hua Yu¹, Ji-Xiang Chen¹, Xiang-Hong Wang¹, P.A.W. Robertson², B. Austin² and Huai-Shu Xu¹. Heterotrophic bacteria flora in aquaculture environment around Qingdao. *Journal of the Ocean University of Qingdao* (English Version) (in press)
2. Zong-Jun Du¹, Yun Li¹, De-Hua Yu¹, Xiang-Hong Wang¹, Ji-Xiang Chen¹, P.A.W. Robertson², B. Austin² and Huai-Shu Xu¹. Heterotrophic bacteria flora in industrial and clean environment around Qingdao. *Journal of the Ocean University of Qingdao* (English Version) (in press).
3. DU Zong-jun¹, WANG Peng¹, LI Yun¹, P. A. W. Robertson², B. Austin². Isolation and identification of two bacteria producing agarase. *Marine Sciences*, 2002, Vol. 26(3): 1~3. (In Chinese)
4. DU Zong-Jun, WANG Xiang-Hong, LI Yun, CHEN Ji-Xiang Research advances on agarases *Microbiology*. (In Chinese) (in press).
5. Chen Ji-xiang, Liu Shuang, Li Yun, Wang Xiang-hong, Du Zong-jun, Yu De-hua, Ji Wei-shang, Xu Huai-shu Purification of an extracellular protease from *Vibrio anguillarum* and its physicochemical properties. *Journal of Fishery Sciences of China* (In Chinese) (in press).
6. Yu Yong, Li Huirong, Li Yun, Ji Weishang, Xu Huaishu Screening of bacteria to degrade the organic pollutants in environment of shrimp farming. *Journal of Ocean University of Qingdao* (In Chinese) (in press).
7. WANG Bao-kun, YU Jun-hong, LI Yun, JI Wei-shang, XU Huai-shu. Isolation and Identification of Pathogen (*Vibrio harveyi*) from Sea perch (*Lateolabrax japonicus*). *Journal of Ocean University of Qingdao* (In Chinese) (in press).
8. Sparagano, O.A.E., Robertson, P.A.W., Xu, H.-S., Li, Y., Sheng, J., Yu, D.-H., Yu, Z.-H., Zheng, G.-L., McInnes, J., Purdom, I. and Austin, B. (2000). Molecular techniques used to identify *Vibrio* species, with specific emphasis on the genomic analysis of the 16S and 23S rRNA genes. *Proceedings of the International Symposium on Marine Biotechnology*. Ocean University of Qingdao Press, pp. 267-270.
9. Sparagano, O.A.E., Robertson, P.A.W., Purdom, I., McInnes, J., Austin, B., Li, H., Yu, D.-H., Du, Z.-J. and Xu, H.-S. (2002). PCR and molecular detection for differentiating *Vibrio* species. *Annales of the New York Academy of Science* (in press).
10. Robertson, P.A.W., MacInnes, J., Sparagano, O.A.E., Purdom, I., Li, Y., Yu, D.-H., Du, Z.-J., Xu, H.-S. and Austin, B. 2001. Bacterial diversity in the marine environment around Qingdao. *Journal of the Ocean University of Qingdao*, (in press).

Another manuscript dealing with biodiversity and its impact on biotechnology is in preparation, and should be submitted during 2002.

Æ Training projects - please provide a **full** account of the training provided. This should cover the content of the training, arrangements for selecting trainees, accreditation, etc.

- **Year 1 - First training session.** Professor H.S. Xu and Associate Professor Yun Li visited Heriot-Watt University from June 27th to plan the project and receive training. Detailed discussion centred on the choice and location of sites to be sampled, the sampling season, methods for the collection and processing of the samples, the recovery and purification of bacteria, and the identification methods to be used. Agreement was reached on the physico-chemical parameters to be recorded. Thereafter, the Chinese visitors worked extensively on a one-to-one basis with the Research Associate, Dr. Robertson. This intensive training period dealt with all the practical aspects concerned with the acquisition and examination of samples, including preparation of media and statistical examination of the data. Samples were collected from a beach at Dunbar and Granton. Certificates were issued at the end of the training period on August 6th.
- **Year 2 – Second training session.** The two Darwin Scholars, De Hua Yu and Zong-Jun Du visited HWU during November for a training period concerned with bacterial identification methods. With one-to-one supervision from Professor Austin and Dr. Robertson, the Chinese visitors used cultures – collected during the research project in China – and covered the use of commercial bacterial identification systems (predominantly the BIOLOG-GN system), the use of serological procedures (latex agglutination, Western blotting), protein profiling, and DNA sequencing. Certificates were issued at the end of the training period (copies attached).

Æ Did any issues or difficulties arise in running and managing this project?

The greatest difficulty was the untimely death of Professor Huai-Shu Xu early in Year 3 of the project. However, we are grateful for Associate Professor Yun Li, who assumed the role of local co-ordinator. Another difficulty reflected the longer than anticipated time necessary for the Chinese scientists to acquire passports and visas. This caused delays in some of the activities, notably in Year 1. Finally, it should be emphasised that problems were encountered in transferring monies to China. Often, the local bank failed to inform the OUQ that the monies had been received. On one occasion, there was denial that the monies had actually arrived in Qnigdao.

7. Project Impact

Æ To what extent has the project assisted the host country to meet its obligations under the Biodiversity Convention, or to what extent is it likely to do so in the future? Please take account of the following in preparing this section of the report:

Æ The way in which research findings have been used to address biodiversity objectives. What actions have been taken, or are expected to be taken, as a result of the project? How will these contribute

towards the conservation of biodiversity in the host country concerned?

Although data are still being analysed, it should be emphasised that the research project has already highlighted weakness in the knowledge base of OUQ. Such weaknesses are still being tackled. Nevertheless, the Chinese scientists have been active in informing Chinese scientific societies, the Shandong Province and central government about the research and, in particular, about the problems of pollution on aquaculture (which is a major industry particularly in Shandong Province) and on human health. An example, which is being used repeatedly, concerns the coastal environment in the vicinity of a coal-fired power station in Qingdao (= Site C). This location also receives effluent from a dyehouse. The result is that the coastal water adjacent to the outflow is acid (pH 1.0) and is coloured. Moreover, the site is within a few kilometres of aquacultural facilities. This perceived problem to the quality (and indeed credibility) of aquacultural produce, much of which is the source of hard-currency being sold to Japan, is forcing the Chinese to act. The data from this project is crucial to the arguments which are being developed. Another strand in the argument concerns the adverse impact of pollution on the biotechnological potential of the coastal marine microflora. However, it will take until summer to complete the ongoing research into this aspect (this work is additional to that of the Darwin Initiative Project).

^x The extent to which training provision has improved the capacity of the host country to conserve biodiversity in the future, and the extent to which the training has addressed real skill needs. Information should be provided on what each student/trainee is now doing (or what they expect to be doing in the longer term), and the extent to which their skills are being used in a positive way to promote biodiversity conservation in the host country.

We have been able to fill some significant gaps in the knowledge base of our Chinese collaborators. Thus, we are convinced that more meaningful microbiology will be carried out in the future. OUQ is committed to bacterial taxonomy/biodiversity, and I envisage that they will focus on this area of research. In terms of destinations, Yun Li was promoted from Assistant to Associate Professor during the period of the contract. Undoubtedly, she will continue with this type of research. Also, Associate Professor Xiao-Hua Zhang will be engaged in this topic, once she returns to Qingdao. Of the two Darwin Scholars, Zong-Jun Du is now a Lecturer in the Department of Marine Biology, and has indicated that he will collaborate with Associate Professor Yun Li on projects in marine microbiology. De Hua Yu has been appointed to an

administrative position in OUQ, and will unfortunately not continue as a laboratory-based scientist.

- Æ The wider impacts of the project in terms of the level of collaboration achieved between UK and host country institutions, and the prospects for greater joint working/information exchange in the future. To what extent has good collaboration been achieved?

HWU and OUQ are pleased with the collaboration. We have succeeded in producing papers, and interacting closely on a topic of mutual interest. We have learnt a lot from each other, and will try for collaborative projects in the future.

8. Sustainability

- Æ Did the host country institute(s) contribute resources to this project (these may have been provided in-kind, for example staff, materials etc)?

Yes

- Æ If so, what is the monetary value of the resources committed to the project by the host country institute(s)?

[REDACTED]

- Æ To what extent was Darwin funding a catalyst for attracting resources (including in-kind contributions) from other sources? Please provide details on the other sources from which resources were secured for this project.

[REDACTED] was given by Aquaculture Vaccines Limited to extend the work, especially connected with aquaculture facilities.

The Chinese Ministry of Education have awarded [REDACTED] Yuan to OUQ to expand/develop the research and educational activities in marine science.

UNESCO have provided funding for training in marine biodiversity (to scientists from developing countries)

Professor Xu was awarded a research contract by the Chinese Ministry of Science and technology (title of project: Aquaculture techniques and disease control of turbot).

- Æ What is the monetary value of resources generated for the project from other sources (please provide an estimate for each funding source)?

[REDACTED]

Æ To what extent is work begun by the project likely to be continued in the future (if this is relevant - some projects may come to a natural end at completion)? This is more likely to be relevant for research-based projects.

With the funding from the Chinese government and UNESCO, the work is continuing under the direction of Associate Professor Yun Li.

Æ Has the project acted as a catalyst for other projects/initiatives in the host country? Is it likely to do so in the future?

The answer to this question is yes for the reasons given above.

9. Outcomes in the Absence of Darwin Funding

Æ Had Darwin funding been unavailable for the project, what would have been the most likely outcome:

Æ The project would not have proceeded?

Æ Had this project not been undertaken, how would the users/beneficiaries of the project have met their requirements? Would other organisations/ initiatives have been able to meet their needs (at least to some extent)?

Probably not.

10. Key Points

Æ What would you identify as the key success factors of this project?

- The opportunity for collaboration on an important issue with a high quality academic institution in a developing country.
- The opportunity to present the scientific data at a Workshop to senior Chinese scientists.
- The opportunity to publish the results of the research.

Æ What were the main problems/difficulties encountered by the project?

The untimely death of Professor Huai Shu Xu.

Æ What are the key lessons to be drawn from the experience of this project? Please try to provide as much information on this point as you can so that others can learn from the experiences of your project.

- Patience is required, and timetables can slip because of problems outwith the control of the participants. For example, the initial timetable anticipated the speedy acquisition of passports and visas for the Chinese — this was not the case.
- Foreign currency. The Chinese scientists needed immediate access to foreign currency. There was a delay whilst this was arranged. (the foreign currency was needed to cover transportation and accommodation costs in the UK)
- Although the participants communicated in English, the level of language proficiency was varied. Some confusion resulted from misunderstandings resulting from the use of incorrect or inappropriate English. The biggest problem was with regard to ambiguities in the scientific data.
- In terms of the science, the salient message is do not assume that the overseas participants are competent in basic techniques — we needed to cover even the most elementary procedures.
- Accounting procedures do not always reach the high standard of the U.K.

Æ Does the experience of this project imply a need to review arrangements for developing and managing projects funded as part of this Initiative?

No.

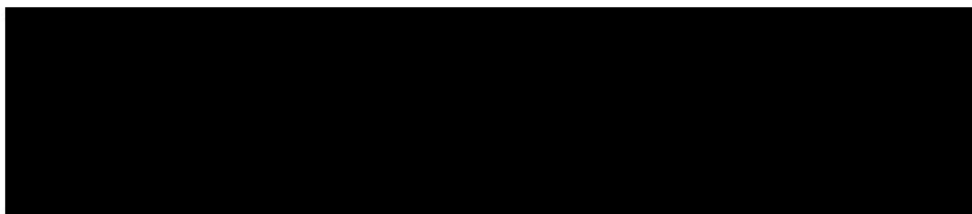
11. Project Contacts

To assist future evaluation work, please provide contact details (name, current address, tel/fax number, e-mail address), for the following:

Æ UK project leader (and other key UK staff involved in the project)

Professor B. Austin

Department of Biological Sciences



Professor F.G. Priest

Department of Biological Sciences



Dr. J.G. Burgess

Department of Biological Sciences



Æ Host country project leader/co-ordinator (and other key people involved in the project at the host country collaborating institute)

Director: Prof. Huai-Shu XU

Associate Prof. Yun LI

Æ 'End users' for the output produced by the project in the host country (e. government departments, agencies, universities, local communities etc)

Ocean University of Qingdao; Government of Shandong Province; Chinese Ministry of Education and Science

Æ Project trainees/students

Main Researchers: Zong-Jun DU (= Darwin Scholar); De-Hua YU (= Darwin Scholar); Xiang-Hong WANG

Post doctoral Fellow:

Ji-Xiang CHEN (part-time)

Ph.D students:

Peng WANG (part-time)

Gui-Li ZHENG (part-time)

Wan-LI GOU*(part-time)

Ju SHENG (part-time)

Graduate students:

Shuang LIU (part-time)

Yong YU* (part-time)

Bao-Kun WANG* (part-time)

Æ Other project beneficiaries

None identified.

Æ Other key players involved in the funding/operation/utilisation of the project.

None identified.

**PLEASE REMEMBER TO ATTACH COPIES OF ALL DOCUMENTATION
PRODUCED BY THE PROJECT IE. REPORTS, PAPERS,
MANUALS GUIDES, CONFERENCE/WORKSHOP PROCEEDINGS
TRAINING MATERIALS ETC**

Ref: 9120/FORMS/9120-FRS