TDR/BCV/93.1

REPORT ON AN INFORMAL CONSULTATION ON DEVELOPMENT OF ENTOMOPATHOGENS FOR VECTOR CONTROL:

CURRENT STATUS AND FUTURE PROSPECTS.

UNDP/WORLDBANK/WHO
Special Programme for Research and Training in Tropical Diseases
(TDR)

45400 ja

Geneva, Switzerland, 17-19 September 1992

REPORT ON AN INFORMAL CONSULTATION ON DEVELOPMENT OF ENTOMOPATHOGENS FOR VECTOR CONTROL: CURRENT STATUS AND FUTURE PROSPECTS

Table of Contents

DEVELOPMENT OF ENTOMOPATHOGENS FOR VECTOR CONTROL: CURRENT STATUS AND FUTURE PROSPECTS

The meeting was opened by Dr B. Dobrokhotov, Secretary, Steering Committee on Biological Control of Vectors (SC-BCV), who welcomed the participants and explained the objectives of the consultation. He emphasized the need for further improvements of the biological methods for vector control. He suggested that the group make recommendations on the development and practical use of entomopathogens in vector control programmes in endemic areas, in particular for the control of malaria.

Dr Lüthy provided participants with an update on the activities and research projects of SC-BCV over the past decade. SC-BCV has initiated and accomplished a wide range of studies on a variety of subjects including microbial control agents and their action, parasites, predators and competitive snails and genetic engineering. Many biological agents in these groups were investigated for their potential as vector control agents. As a result of extensive search, isolation, characterization and evaluation, two new promising microbial entomopathogens have been isolated and developed for vector control. Dr Lüthy emphasized the need for further research to broaden the use and application of these and other entomopathogens in vector control programmes, especially in tropical endemic areas.

1 STRATEGIES IN THE MANAGEMENT OF RESISTANCE TO MICROBIAL AGENTS

1.1 Current Status

Although *Bacillus thuringiensis* (*B.t.*) strains have been utilized in agricultural pest control (primarily Lepidoptera) for more than twenty years, only one insect species (*Plutella xylostella*) has become resistant to it. *Plutella* resistance to *B.t. kurstaki* (*B.t.k.*) has been reported from Thailand, Philippines, Hawaii and Florida. Available field and bioassay data have demonstrated the development of resistance (at 2-20 times the standard dosage) to all commercial strains of *B.t.k.*. Several laboratory studies have demonstrated development of resistance in laboratory insect colonies that had been "stressed" for multiple generations with *B.t.k.* *Plodia spp.* (Indian meal moth) and *Heliothis spp.* have been shown to develop resistance to *B.t.k.* In the case of *Heliothis spp.*, resistance to a single toxin challenge developed after 7 laboratory generations.

In all cases of Lepidoptera resistance, after removing selection pressure for several generations, host populations regained susceptibility to *B.t.k.* toxins. The only reported case of resistance to *B.t.israelensis* (*B.t.i.*) in mosquitos is that which developed in *Culex quinquefasciatus* and *Aedes aegypti* when subjected to *B.t.i.* at sub-lethal doses for several generations in the laboratory. As with Lepidoptera species, the mosquitos regained susceptibility to *B.t.i.* when removed for several generations from *B.t.i.* stress.

1.2 Potential critical assessment

These reports would indicate that Lepidoptera and mosquito larvae may develop resistance to the multiple toxins present in B.t.k. and B.t.i. (Cry IVA, Cry IVB, Cry IVC, Cry IVD, Cyt A), but that the rate of development is much slower than to synthetic chemicals. Resistance obtained in laboratory colonies has been transient. The risk of resistance to B.t.i. or B. sphaericus developing in natural field populations would appear to be low. Although B.t.i. has been widely used in operational control programmes against mosquitos and blackflies, no incidence of resistance or decreased susceptibility has been reported to date in the target species.

It should also be emphasized that Plutella spp. which exhibited some resistance to B.t.k. were readily susceptible to B.t. aizawai toxins. Use of pesticide rotation using B.t. strains and chemical insecticides for mosquito or blackfly control would further reduce the potential for the development of resistance to both groups of control agents.

The temporary development of resistance to B.t. strains has been investigated and in a preliminary study it has been shown to be associated with a change or effect on the receptor sites of midgut epithelial cells. Receptor sites that were not susceptible to B.t.k. were susceptible to toxins present in other B.t. strains.

1.3 Methodology/Techniques

Insect resistance studies undertaken to demonstrate or explain resistance mechanisms in mosquitos or blackflies must be based on standard protocols developed for both laboratory and field studies. In order to ensure that laboratory results can be reproduced and that there is a sufficient host gene pool, insect colonies (for example of Aedes aegypti, Culex quinquefasciatus) must be available at a variety of testing laboratories. International standards for microbial agents must be available and utilized for comparison in all studies. At the present time these standards are IPS 82 for B.t.i. and SPH 88 for B. sphaericus strain 2362.

Limited short-term studies involving the development of resistance to B.t. and B.s. or other microbial agents are currently in progress. Further elaboration of the mode of action of resistance would involve a medium-term to long-term study, with no guarantee of success. As we have seen in previous laboratory experiments, resistance to B.t.k. or B.t.i. can be a transient event and the study of resistance would require maintenance of B.t. resistance in a host colony. One of the goals of resistance studies should be the collection of reliable baseline data for those species that would form the basis for future comparison with field collected susceptible or resistant populations.

2 INTEGRATED MANAGEMENT OF VECTORS AND THE ROLE OF MICROBIAL CONTROL AGENTS

2.1 Current status

A variety of environmental, economic and other concerns have necessitated the development and use of an integrated approach to vector control. Where the use of insecticide is reduced or eliminated, other interventions such as environmental management and biological control play an increasing role. The decrease in the availability of effective insecticides due to the emergence of resistance in target species and to the non-renewal of registration of some insecticides has given further impetus to the development of alternative means of vector control. The prohibitive cost of developing new products is another important reason for using less chemical insecticide.

The integrated approach to vector control seeks to lower the population equilibrium of mosquitos to levels at which pestiferous activity is minimal and disease transmission is reduced or interrupted, using methods which have minimal adverse effects on the environment. The components of integrated vector control (IVC) include the use of environmental management, biological control as well as the judicious use of chemical insecticides. A comprehensive approach to IVC does not usually include all the possible options. Rather, it incorporates the most effective, complementary and environmentally compatible interventions.

2.2 Critical assessment

Only in the simplest of confined ecosystems, can biological control be used alone for the successful abatement of disease vectors. However, the integration of biological control into the framework of a well-planned IVC programme can have a significant impact on vector populations. Key components of this strategy should include a thorough geographic reconnaissance of mosquito habitats and in-depth understanding of the bionomics of target mosquitos, the ecosystems they occupy and the organisms intended for use as biological control agents. Regular evaluation of control methods through the monitoring of larval and adult mosquito abundance is essential for guiding the direction and intensity of IVC programmes.

One of the most important components of IVC will be the regulating role of natural enemies in the reduction of mosquito populations. The use of microbial control agents will promote the conservation of natural enemies which is essential for implementing long term, cost-effective vector control programmes and minimizing treatments.

In addition to their effectiveness, the value of bacterial agents is in their high level of specificity. As the influence of bacterial agents on the non-target organisms is virtually nil, a full component of natural enemies in the ecosystem is preserved.

The role of natural enemies in conjunction with the use of bacterial control agents provide a strategy of spontaneous biological control resulting in much reduced population density of vectors in relation to their reproductive potential. This natural control system operates through a large number of abiotic and biotic factors including pathogens (virus, bacteria, fungi, protozoa), invertebrate predators and parasites (nematodes, crustacea, insects) and vertebrate predators (fish).

Microbial control agents, particularly Bacillus thuringiensis (H-14) and B. sphaericus are effective mosquito control agents that have minimal or no impact on the complex of natural enemies and could usefully be included in an integrated programme for the control of mosquitos.

2.3 Integration of bacterial larvicides with other biocontrol agents

In addition to the integration of microbial control agents into classical environmental methods (source reduction, environmental modification, personal protection), a promising area that warrants further research and attention is the combined use of microbial control agents with other biological methods. In particular, the combined use of bacterial agents with other biocontrol agents such as predators, parasites and other pathogens can bring about a rapid reduction of target populations, with the bacterial agents providing immediate control and the other biocontrol agents sustaining the reduction of rebounding mosquito populations. In the case of macroinvertebrate and fish predators there is a characteristic time lag between inoculation into a habitat and build up of sufficient numbers to achieve control. Bacterial larvicides, however, provide rapid control of initial populations without destroying the complex of natural predator species and other non-target organisms. In situations requiring inundations and stocking, fish and other larvivorous organisms can be introduced after or during treatment with a bacterial agent and in this way, immediate as well as sustained control of vectors, without further treatments, can be achieved.

These IVC strategies can be employed in a variety of habitats including small ponds, rice-fields and large water storage containers.

3 STRATEGIES IN THE TRANSFER OF MODEL PROJECTS TO ENDEMIC REGIONS

3.1 General considerations, actual status and critical assessment

A vital component of successful vector control programmes in the tropical or temperate climatic zones is interdisciplinary collaboration between various specialists under the aegis of health services and other vector-borne disease control agencies. It is important that relevant data on the biology, ecology and epidemiology of target organisms and diseases are gathered. In the interests of promoting community participation and minimizing costs, appropriate methodologies and techniques should be employed.

At all stages of planning but particularly in the implementation phase, close cooperation between the national authorities, scientists, industry and the public is a prerequisite for the success of vector-borne disease control programmes. An early exchange of information and ideas between the different sectors in tropical and temperate climatic zones is vital for further development of vector control programmes, especially when new methodologies such as the use of microbial control agents in integrated control programmes are being considered. The intention in transferring model projects, for example from temperate climatic zones to endemic zones, is not to duplicate a successful programme in all details but rather to apply proven principles and logistics. A strategy for vector control must take into consideration local conditions and ecological and epidemiological factors. It is not possible to apply a single model to all types of local situations. The aim therefore should be to devise a comprehensive control strategy that takes into account the particular ecological, epidemiological and sociological aspects of each situation.

3.2 Required steps

As pointed out above, the transfer of knowledge and experience has to take place on an interdisciplinary basis, at different levels.

a) As decision makers, administrators and representatives of the local authorities should be involved in the transfer of ideas and principles. They should be convinced that the proposed strategy will be successful and accepted by the public. The specific advantages of the microbial control agents have to be highlighted. The efficacy and environmental safety of the microbial control agents provide good arguments. Educational media can be used to explain the objectives of the campaigns and to provide basic information on vectors and vector control.

b) There are various levels at which scientists need to cooperate. They have to develop a general outline of the implementation of the programme at different places in the region. The procedure for testing new agents needs to be standardized using laboratory bioassays; the effective and optimum dosage of a product against various mosquito species needs to be assessed in small-scale field tests; ecological, entomological and epidemiological data need to be assessed. It is important that baseline data be gathered before the implementation of any large-scale evaluation project.

c) Effective community participation in the programme will necessitate comprehensive instruction on the biology of the vector and on straightforward methods of control, the involvement of various community groups as volunteers and the linking of mosquito control with other activities such as income-generation and environmental sanitation projects. The suitability of microbial control agents for community use has to be highlighted, in particular its safety for humans and the environment.

d) Good coordination between industry, science and health services is essential in the implementation of successful programmes. Once a product is developed, experience regarding its use in the field needs to be shared in order to make further improvements. The special needs of each situation have to be considered in order for it to be successfully marketed and used. The design of the product must suit the integrated approach of the programme.

3.3 Methodology - tools for the transfer of principles of successful programmes

The transfer can take place in various ways:

a) on a bilateral basis, through regular site-visits to research and scientific organizations and national and local organizations;

b) in national and international workshops;

c) through cooperation between international agencies such as WHO/TDR and national organizations;

d) through training courses where scientists can exchange information and experience.

4 STEPS TOWARD FURTHER DEVELOPMENT OF BACILLUS BREVIS AND CLOSTRIDIUM BIFERMENTANS MALAYSIA

4.1 Bacillus brevis

4.1.1 Current status

To date, 41 isolates of this bacterium have been tested and all showed some degree of molluscicidal activity against the snail *Biomphalaria glabrata*. The activity assessed was that of cultures in the pre-spore (< 1% sporulations) stage. Death occurred with cells after 3-5 days and with the toxin extract after only 6-9 hours.

Safety. No studies on mammalian toxicity have been carried out.

Non-target organisms (NTOs). Several different species of adult snails were exposed and they survived. Tests on other aquatic organisms are planned for the future.

Physiology and molecular biology. Studies on the genetic (clonal) composition of the species and the toxin associated with cell wall fraction of the cultures are in progress. The toxin is not water soluble and it is absent from the supernatant. Toxin can be extracted from the cell. The toxin, as part of the cell, is heat stable and is sensitive to protease and lysozyme but not RNAse or DNAse.

Fermentation techniques

a) Primary powders have been made and are stable for as long as six months. Addition of one of eleven sugars (e.g. fructose) is necessary for production of molluscicidal toxins.

b) Toxin apppears as early as 9 hours and disappears at about 20 hours (or 1% sporulation) of fermentation.

4.1.2 **Benefits**

a) To date this is the only species of *Bacillus* known to have molluscicidal activity.

b) There is potential for the development of a quick industrial fermentation process within a year or so.

4.1.3 **Prospects**

a) Its toxicity is moderate but can be enhanced by improvement in fermentation techniques and biotechnology.

b) The activity of 3 Ghanian strains has been demonstrated in two laboratories and material will be supplied to other laboratories for broader testing.

4.1.4 **Short-term goals**

a) To characterize toxin and biogenesis of toxin.

b) To complete initial fermentation development.

c) To continue some screening for other potential *Bacillus* molluscicidal strains.

4.1.5 **Intermediate goals**

a) To enhance toxicity.

b) To examine action on other vector snails.

c) To carry out safety and NTO studies.

d) To initiate small-scale field trials.

4.2 *Clostridium bifermentans malaysia*

4.2.1 **Efficacy**. Demonstrated against mosquitos and *Simulium*, (*Anopheles* most susceptible followed by *Culex* and then *Aedes*).

4.2.2 **Safety**. Toxin is soluble and excreted after lysis of the cells. Field testing of cells per se is safe.

4.2.3 **Non-target organisms (NTOs)**. In trials on several NTOs (including fish and aquatic NTOs), it has been shown to be safe except for *Chaoborus* which are also pests in some areas of the world.

4.2.4 **Physiology and molecular biology**. Toxin characterization is on-going. Not homologous with *B.t.i.* and *B.s.* toxins.

4.2.5 **Cytopathology**. The ultrastructure and the mode of action appear to be different from *B.t.i.*. A gene library has been set up.

4.2.6 Benefits

a) It is the second bacterial toxin found to be active against Simulium

b) It has the same level of toxicity for mosquito larvae as B.t.i. and B. sphaericus.

c) It lacks mammalian toxicity.

d) It possesses good specificity.

e) Taxonomically, it is distant from medically important Clostridium.

4.2.7 Drawbacks

a) It is heat and protease labile (when lyophilized stable for one year).

b) It has a bad name; before subjecting it to field trials, regulatory agencies such as USEPA will need to be consulted.

c) As it is completely anaerobic, there may be difficulties in fermentation development.

d) It will probably be necessary to find a new host for toxin genes.

4.2.8 Short-term goals

a) To isolate the responsible gene for toxin(s).

b) To determine precise activity against Similium.

c) To characterize and isolate toxin(s) protein(s).

d) To identify new hosts as carriers for toxin(s) gene(s).

4.2.9 Intermediate goals

a) To complete safety testing.

b) To further assess stability of toxin(s).

4.2.10 Long-term goals

a) To undertake mode of action studies.

b) To integrate toxin(s) into an easy growing non-pathogenic organism.

c) To undertake field trials after clearance from EPA and other regulatory agencies.

5 THE SEARCH FOR AND DEVELOPMENT OF NEW MICROORGANISMS FOR VECTOR CONTROL

5.1 Current status

Currently we have two highly mosquitocidal agents of Bacillus pathogens (B.t.i. and B. sphaericus) and other promising bacterial species (C.b.m. and B.t. medellin) under study. Although these strains are highly effective, we still need to continue searching for new isolates with mosquitocidal activity especially in areas in which surveys have not previously been conducted. In order to be able to cope with any resistance to the toxins of currently available bacteria that may emerge in target species, it will be necessary to isolate bacterial strains which differ significantly from those currently in use in their potency, in their toxins or in their modes of action.

5.2 Critical assessment

Methods that are used for isolation of spore-forming bacteria often do not allow for the possibility of discovering non-spore-forming species and initial screening of new isolates does not usually include a broad spectrum of target species such as blackflies and others.

In recent searches, fungi have been neglected in terms of isolation and development efforts. Another neglected aspect of microbial control agent development is small-scale fermentation (5 litres) to determine the feasibility for further commercial development.

5.3 Required steps for further development

The following steps are listed as short-term goals:

a) To continue the search for new isolates;

b) To broaden the scale of isolation techniques (utilizing unpasteurized samples and selective media);

c) To broaden the screening system (minimum of Aedes aegypti, Culex and Anopheles sp. and also additional screening on Simulium sp.);

d) To scale-up fermentation (volume more than 55 litres);

e) To ensure a close association between laboratory, field testing and pilot fermentation.

6 FUTURE RESEARCH IN THE FIELD OF MOLECULAR AND GENETIC ENGINEERING AND MODE OF ACTION

6.1 Current status

A considerable amount of research is being undertaken in this area in several laboratories throughout the world. Among the topics receiving current research attention are the Cry IV toxins of B. thuringiensis (H-14), and in particular the interaction between Cry IV toxin active against mosquitos or blackflies. Regions of the toxin molecules responsible for toxin binding and activity are being delineated. In the case of the B. sphaericus toxin, current studies are aimed at understanding the function of each binary toxin protein in the mode of action and the host range. A toxin of ca. 110 kDa has recently been isolated, and its host range and stability are under study.

In the short term, it is clear that the greatest progress will be made through extending the above studies, as well as through the cloning and expression of genes from strains of other serotypes of B. thuringiensis with activity toward mosquitos and blackflies, for example B. kyushuensis, fukuensis, darmstadiensis, morrisoni, and the CryIIA proteins. Toxin genes in strains of B. sphaericus encoding components showing higher toxin activity also need to be cloned.

In the foreseeable future, receptors for both the Cry IV toxins of B.t.i. and for B. sphaericus toxins should be identified and isolated from mosquitos and blackflies and characterized with regard to receptor affinity and number. Receptors in highly susceptible insects should be compared with those in poorly sensitive or resistant insects, in order to understand the cause of reduced susceptibility of these hosts. The mode of action of both B.t.i. and B. sphaericus toxins must be further elucidated. The significance of differences in activation products among B.t.i., Cry IV proteins should be determined, with a possible comparison of activation by cloned mosquito trypsin with mammalian trypsin.

7 RESEARCH AND DEVELOPMENT OF RECOMBINANT VECTOR CONTROL AGENTS - REGULATORY CONSTRAINTS

In the past decade, two microbial control agents, B.t.i. and B. sphaericus have attracted a great deal of attention for the control of mosquitos and blackflies. B.t.i. has been used for a number of years in mosquito and blackfly control programmes and B. sphaericus has recently been commercialized. In general, neither of these materials are persistent and they settle outside the feeding zones of mosquito larvae. It is possible that these drawbacks can be overcome through genetic manipulation.

In recent years, the toxin genes alone or in combination have been cloned in blue-green algae Anacystis nidulans. However, some of these algae although ingested are not digested. What is needed therefore, is cloning in an organism which persists in mosquito larval feeding zones and which is ingested and digested. Another area for recombinant technology is to increase the floatability of B.t.i. and B. sphaericus by introducing the gvp genes of blue-green algae into the bacterial cells to produce gas vacuoles for easy floatage. B. sphaericus will be a good candidate for use in these studies because of its somewhat greater persistance and recycling potential.

7.1 Deliberate release

The consultation addressed the problem of deliberate release of transformed organisms into the environment. This problem needs to be solved at the national and international levels, before genetically altered organisms are deliberately released. The process of "risk assessment" lies at the root of this controversy. A great deal of research is needed in order to fully assess the risk. When a genetically-altered organism is released into the environment, there are a number of possible outcomes:

a) the microorganism cannot survive in the new environment due to competition and other disadvantages;

b) the microorganism establishes itself as anticipated and performs the desired function;

c) the microorganism becomes a "rascal" after release and affects the environment in an unforeseen and unpredictable deleterious manner.

It is imperative that requirements for the deliberate release of altered organisms into the environment be drawn up after rational consideration of the scientific basis of the study with respect to the nature of the microorganism involved, the site of release and assessment of the potential risks and benefits derived from the release. The consultation discussed the need for preliminary dialogue with regulatory agencies to get a reading on the extent of regulatory requirements for release of genetically-engineered organisms.

In the meantime, it is desirable to continue limited studies on recombinant DNA technology with the following objectives in mind:

a) To improve toxin yield in B.t.i., B. sphaericus and others;

b) To broaden the target activity range;

c) To construct an organism with favourable environmental attributes.

8 FORMULATION

In the discussion on further development and use of entomopathogens, it was emphasized that tailor-made formulations are needed for the control of vectors in specific situations. Currently available formulations do not provide cost-effective control of anopheline vectors of malaria. In most of these formulations, the toxin particles settled outside the feeding zone of mosquito larvae rather quickly. However, preliminary data on formulating B.thuringiensis (H14) and B. sphaericus in surface spreading film showed increased activity against both Culex and Anopheles larvae. Toxin particles in the oil film stayed at the surface for longer periods than toxin particles in the powder formulation. The activity of both pathogens was increased 5-7 fold against Anopheles larvae by simple formulation techniques. This is indeed a notable increase in efficacy which could not be obtained by other techniques.

Current information derived from the use of controlled-release formulations was critically reviewed and analyzed. It was noted that these formulations (briquets, tablets and other compositions) are quite effective in confined mosquito breeding sources. Surface spreading film formulations will be more suitable for the treatment of large expanses of water where anophelines breed for limited periods.

The group, however, identified the need for promoting simple and traditional technology for developing tailor-made formulations. Simple approaches in solving formulation problems will be more cost-effective and acceptable to the community than sophisticated formulation technology.

The final goal should be to develop and promote simple formulation technology through the implementation of systematic applied research and to promote and facilitate links between industry scientists, and specialists in the various endemic countries.

9 BASIC RESEARCH

The group discussed the need for research in some new areas which could expand the options of new strategies against vectors. Of immediate interest were two areas which were considered as having some potential for research.

9.1 Viruses (in the context of genetic engineering)

So far, in agriculture, the success of viruses as biological control agents has been extremely limited, with the exception of a few baculoviruses which have been commercialized and used on a small scale. Difficulties in virus culture, storage problems, high cost of production and restricted host range have prevented their widespread development.

However, this situation might change as advances in genetic engineering technology are made. Indeed, the discovery of insect, or more precisely mosquito, virus strains which are specific, have high infectivity potential in vectors and at the same time, capability of transformation, could lead to the development of a new group of infectious control agents, through the incorporation and expression of bacterial and other toxins using genetic engineering technology. With these techniques it might be possible to reach mosquito adults and develop a self-sustaining strategy.

This is indeed an attractive possibility but does not deserve high priority as long as no such mosquito viruses are known. However, the possibility should be borne in mind and the genetic engineering aspects researched, if and when such viruses are discovered.

9.2 Attractants and Semiochemicals

Mosquitos are attracted to their hosts through odours in combination with physical stimuli. These cues have been poorly defined. Similarly, the biology of oviposition attractants is poorly understood at the chemical or physiological level. The effects of odour combinations (blends) have been studied recently in host finding by phytophageous pests, but not in human disease vectors with the exception of one or two groups. Development in this area could lead to novel attractants for oviposition traps or perhaps added to breeding sources along with microbial larvicides or other larvicidal agents.

Immediate application of this technology is not expected in the near future and therefore does not need high priority at this time. A great deal of basic research is needed to gauge the potential of these approaches in vector control programmes.

10 RECOMMENDATIONS

10.1 Integrated Vector Control

To promote a basic understanding of integrated vector control in developing countries with greater emphasis on vector bionomics and ecosystem analysis.

To investigate the combined role of natural enemies and bacterial control agents for the development of cost-effective vector control technology in a variety of habitats including those of anopheline mosquitos. Conservation of natural enemies through the use of selective larvicides (e.g. bacteria) should provide affordable strategies.

10.2 Transfer of model projects

To promote and facilitate the exchange of information and ideas between cooperative programmes in order to maximize benefit.

Through appropriate channels, WHO should publicize the advantages and safety attributes of microbial control agents such as *B.t.i.* and *B. sphaericus* and this information should be incorporated into the guidelines for the control of relevant vectors and pest species.

10.3 Search and isolation

To continue the search for, isolation and evaluation of entomopathogens especially in areas where such a search has not been carried out before. To test such isolates against a broad spectrum of mosquitos and other vectors and to send the most promising isolates to appropriate collaborating laboratories for further testing and characterization.

To carry out scale-up fermentation studies on promising agents to show their potential for commercial production.

10.4 Molecular and genetic studies

To continue research on the identification and characterization of receptors for B.t.i. Cry IV toxins as well as B. sphaericus toxins.

To study the mode of action of toxins of both bacterial agents with the eventual goal of manipulating them genetically to enhance activity and to broaden the host range.

10.5 Genetic engineering - regulatory constraints

Rather than developing its own guidelines for the deliberate release of transgenic and genetically-altered organisms, WHO/TDR should rely on the guidelines established by EPA, NIH, EEC, OECDE or other national or multinational agencies.

Research projects involving field release of altered organisms in countries without guidelines for such release, should not be supported.

10.6 Formulation

To undertake field evaluations of various formulations with the aim of making the use of entomopathogens for the control of malaria vectors and other species cheaper and more effective.

To establish links between scientists in developed and developing countries to develop and evaluate tailor-made formulations for specific situations.

To collaborate with and seek the assistance of industry in designing and providing formulations for testing and evaluation in endemic areas.

10.7 Resistance management

Since no significant resistance to B.t.i and B.s. has been observed or demonstrated in most natural field populations, it is recommended that resistance studies not be conducted under artificial laboratory conditions until a significant level of resistance has been documented in natural insect populations.

When recombinant B.t.i. and other transgenic entities become available, laboratory studies should be initiated to compare resistance development to these transformed organisms.

WHO should not recommend using any single toxin, recombinant strain until significant research has demonstrated that there would not be a high risk potential for resistance development to single toxins.

10.8 Bacillus brevis and Clostridium bifermentans malaysia

Since both B. brevis and C. bifermentans malaysia are unique bacterial agents pathogenic to snails, mosquitos and blackflies, it is recommended that further studies be continued on these agents to characterize the toxic proteins and biogenesis of such toxins. Further, it is recommended that the toxins of C.b.m. be purified, characterized and the gene(s) responsible for toxin production be identified and cloned into a suitable organism which can be easily fermented.

Samples of B. brevis active isolates should be produced in sufficient quantities and provided to other laboratories for testing against Biomphalaria glabrata and other snail species.

LIST OF PARTICIPANTS

Dr N. BECKER, Kommunale Aktionsgemeinschaft zur Bekämpfung der Schnakenplage e.V. Ludwigshafen am Rhein, Postfach 210780, DW-6700 Ludwigshafen, Germany

Dr G. CHESTUKHINA, Institute of Genetics and Selection of Industrial Microorganisms, 1 Dorozhny proezd 1, 113545 Moscow, Federation of Russia

Dr E. DAVIDSON, Department of Zoology, Arizona State University, Tempe, Arizona 85287-1501, USA

Dr H. DE BARJAC, Bactéries Entomopathogènes, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France

Dr P. GUILLET, ORSTOM, 2051 Avenue du Val de Montferrand, BP 5045, 34032 Montpellier Cedex 1, France

Dr J.-M. HOUGARD, OCP/OMS, BP 2279, Bamako, Mali

Dr A. KLIER, Unité de Biochimie Microbienne, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France

Dr L. LACEY, European Biological Control Laboratory, BP 4168 Agropolis, 34092 Montpellier Cedex 5, France

Dr P. LUTHY, Mikrobiologisches Institut, Eidgenössische Technische Hochschule Zentrum/LFV, Universitätstrasse 2, 8092 Zürich, Switzerland

Dr M.S. MULLA, Department of Entomology, College of Natural and Agricultural Sciences, University of California, Riverside, California 92521-0314, USA

Dr H. MUSTAPARTA, Department of Zoology, University of Trondheim, N-7055 Dragvoll, Norway

Dr J. RIBEIRO, Department of Entomology, Forbes Building Room 410, University of Arizona, Tucson, Arizona 85718, USA

Dr S. SINGER, Department of Biological Sciences, College of Arts and Sciences, Western Illinois University, Macomb, Illinois 61455, USA

Dr I. THIERY, Bactéries Entomopathogènes, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France

Dr J. VAVRA, Department of Parasitology, Charles University, 128 44 Prague 2, Vinicna, Czechoslovakia

Dr YAP HAN HENG, School of Biological Sciences, Universiti Sains Malaysia, Minden Campus, Penang, Malaysia

OBSERVERS

Dr E. DANKWA and Dr C. CIBULSKY, Abbott Laboratories, Chemical and Agricultural Products Division, 1401 Sheridan Rd, North Chicago, IL, USA

Dr O. SKOVMAND, Novo Nordisk A/S, Novo Allé, 2880 Bagsvaerd, Denmark

Dr B. GALLAGHER and Dr D. BASSAND, Sandoz Agro Ltd, CH-4002 Basle, Switzerland