Sida Evaluation 97/38

Biotechnology Research Projects

Tree Tissue Culture and Proteins/Enzymes as Biosensors

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Department for Research Cooperation, SAREC

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Biotechnology Research Projects

Table of Contents

Tree Tissue Culture

Proteins/Enzymes for Use as Biosensors

Executive Summary

Forests are a critical and valuable national resource for India. Yet there has been widespread destruction and deterioration of forest lands in the country. Only belatedly have government programs been initiated toward afforestation, conservation and restoration of forests. The 1988 National Forest Policy aims at increasing the country's forest cover to a third of the total land area compared to the alarming current low of only about 15%. The forestry sector has been recognised as an important component of sustainable development strategies. The use of biotechnology in the improvement of plants, including forest trees, has been also identified as a national priority under the auspices of the Department of Biotechnology (DBT). It is in this context that the Swedish International Development Cooperation Agency (SIDA) and DBT have jointly supported a research project at the Swedish University of Agricultural Research at Uppsala (Sweden) and the National Chemical Laboratory (NCL) at Pune (India), to develop efficient methods for the large scale clonal propagation of selected species of Indian pines. The success of the project objectives is largely dependent on the research to be carried out at NCL, and should result in the generation of high quality planting material of the selected pine species for afforestation. India, but particularly the NCL, have the scientific ability and technical capacity to benefit from this collaborative project. The research carried out so far has been mainly exploratory in nature and the progress has been rather slow. It must now be accelerated and placed on a more sound scientific footing, so that a production-oriented system can be established during the second phase of the project. Continuation of the project is recommended for another three years, with strategic changes in the scope and focus of the future work and the budget, particularly at the site in India.

1. Introduction

During the past half century, there has been senseless, large scale and often indiscriminate destruction of forest lands throughout the world. The level of deforestation in the developing countries has been particularly devastating. India is no exception to this, and the loss of forest lands in India (forest cover is down to about 15%, in comparison to an ideal of 33% targeted in a National Forest Policy adopted in 1988) is a true national tragedy (per capita forest land in India declined from 0.2 hectare in 1951, to 0.09 hectare in 1991). The understanding of the enormous harmful and long-lasting impact of deforestation on global as well as regional climate and the environment (e.g. global warming and greenhouse effect), and the related loss of biodiversity and wild life habitats, soil erosion and flooding, etc., led to extensive public awareness, concern and debate in the United States in the early 1960's, resulting in the birth of the environmental movement and strong legislative actions to conserve biodiversity and to protect forest and other ecologically sensitive lands by sustainable development strategies, including environmental stability and ecological balance. Similar movements were later started in many countries in Europe, some of which resulted in the formation of political parties, like the "Greens" in Germany and Switzerland. The developing countries, India among them, were slower to react to the environmental and ecological impacts of widespread deforestation. Nevertheless, there is now a robust defense of forest lands by some public groups in India, which are greatly and rightly concerned about the harmful effects of deforestation on the future welfare of the country. These concerns and efforts are fully shared and supported by the Government of India and most of the State governments, which have ministerial level oversight of federal and regional policies and programs not only to safeguard Indian

forest lands and products, but also to enhance them for the people of India, including the nearly 60 million mostly tribal households, particularly their women, who rely heavily on forests for their living. It is also to be noted that the forestry sector is an important and essential component of the concept of sustainable development, a public policy now adopted by most enlightened nations, including India.

The widespread destruction of forest lands in Asia and Latin America, and its impact on global and regional climate, ecology and economies, was viewed with much concern by many international aid agencies and the governments of several industrialized nations. The developing countries contain virtually all of the world's tropical forests which, among other factors, are important carbon sinks and the richest sources of global diversity. One result has been the new focus on the conservation and restoration of tropical forests, which have come to occupy the center stage as a result of the greater understanding and awareness of the enormously harmful consequences of their destruction and disappearance. Another result was the creation by the Consultative Group on International Agricultural Research (CGIAR, World Bank) of the International Center for Research on Agro-Forestry (ICRAF, Kenya) and the Center for International Forestry Research (CIFOR, Indonesia). In addition, several governments have initiated collaborative aid programs with the developing countries to protect forest lands and to provide assistance in afforestation efforts.

2. The Origins of the Indo-Swedish Tree Tissue Culture Project

Improvement of forest species by breeding is an exceptionally difficult and time consuming task, because of the very long breeding cycles, and the fact that the desirable characteristics of trees can not be evaluated until they reach maturity in 15-25

years. Vegetative propagation of elite trees by rooting of cuttings can overcome this problem, but has been found to be impractical because of the high cost, decline of rooting ability with age, and other factors. The quality of available planting material (an estimated annual demand of 5 billion seedlings in India) is poor, resulting in low survival. It has, therefore, been proposed that elite forest trees, which have been selected for their usefulness and high quality, be cloned in large numbers by tissue culture techniques to overcome the problems that are inherent in the breeding of forest species, and to complement and supplement the traditional forestry practices by providing high quality planting material. For a long time, woody species, particularly trees, were found to be very recalcitrant to regeneration in vitro. However, substantial progress in the development of methods for the regeneration of tree species from cultured cells has been made during the past decade. Valuable information is being generated from the study of tissue culture regenerants under field conditions in several countries. During this time, efficient methods for the large scale clonal propagation of eucalyptus, teak and bamboo were developed by scientists at the National Chemical Laboratory (NCL) at Pune, India. In recognition of this, the Department of Biotechnology (DBT), Government of India, established a Tissue Culture Pilot Plant (TCPP) facility at NCL, and charged it with the responsibility to produce tissue culture regenerants of these species in large numbers for field tests and evaluation at forest sites all over India, with the final objective of using such plants in reforestation efforts.

The success of the TCPP enterprise at NCL, and some encouraging early work on *Pinus caribaea* and *P. kesiya* by Nadgauda et al. (1993), led to the development of the collaborative Indo-Swedish Tree Tissue Culture Project, funded jointly by the Swedish International Development Cooperation Agency (SIDA) and the DBT, beginning

in June 1994. The project is result oriented with the expectation that the findings will be of strong practical relevance not only to India but also to other developing countries. The Indian sub-continent has highly varied climate and vegetation. Indian forests contain a wide variety of plants, including many species belonging to the genus *Pinus*. Accordingly, the focus of the research in the project is on three pine species with the following four well defined objectives:

a. Clonal multiplication of *Pinus roxburghii*, *P. kesiya* and *P. caribaea* through micropropagation, organogenesis or somatic embryogenesis from immature zygotic embryos as well as mature tree explants.

b. Growth and scale-up of organogenic or embryogenic cultures of *P. roxburghii*, *P. kesiya* and *P. caribaea* in bioreactors for mass propagation.

c. Development of methods for stimulating maturity in somatic embryos formed in culture.

d. Production of transgenic plants of *Pinus* and *Picea* species. Genetic improvement of forest species by conventional breeding as well as molecular techniques.

Objectives (a) and (b) were to be carried out at NCL under the direction of Dr Rajani Nadgauda, and (c) and (d) at SLU, under the direction of Professor Sara von Arnold.

3. The Purpose and Process of this Review

SIDA and DBT funded the initial project for three years, starting in June 1994. The groups at SLU and NCL have submitted a report of the research carried out during the

period July 1, 1994, to December 1, 1996, along with a proposal to extend the support for another three years (July 1, 1997 to June 30, 2000). In order to make a decision on the proposal for continued support, SIDA has arranged for an independent outside expert to review these documents, site visit the laboratories at SLU and NCL, and make specific recommendations regarding the following:

a. Relevance and importance of the project to India and other developing countries.

b. Economic and social impacts and practical applications of the research.

c. Scientific quality, cooperation and performance of the project.

d. Cost-effectiveness and budgetary justification.

e. Future direction, content and feasibility of the project.

Accordingly, this report is based on extensive discussions during site visits to the laboratories at NCL and SLU, consultations with senior officials of DBT, and the study of project proposals (1992, and 1997) and reports (1996) provided by SIDA.

4. Project Research at the Swedish University for Agricultural Research

The program of research in the laboratory of Professor Sara von Arnold is of a basic nature and covers a broad range of topics related to the physiology, biochemistry and molecular biology of forest trees, particularly *Picea abies, Salix* and some species of *Pinus*. It is a world class research program, that is well funded and directed. The research facilities and infrastructure are excellent. The research is carried out by a number of graduate students and post-docs, in close collaboration with forestry and other faculty. Much of the basic research carried out here is of relevance and benefit to the overall progress and success of the collaborative project with NCL. The research

group at SLU is highly productive, and has made substantial scientific contributions, as evidenced by nearly 25 high quality publications in refereed international journals. The results described in many of these publications are directly related to the SLU's project responsibilities. Of particular interest are the studies on cold hardiness, physiology and molecular biology of rooting, bud quiescence/dormancy, embryo maturation, phenology, gene mapping, genetic transformation, etc.

5. Project Research at the National Chemical Laboratory

The research program at Pune is highly applied in nature, and is focused almost entirely on the development of protocols for the rapid and large scale propagation of forest species. The success of the project depends largely on the ability and ingenuity of the NCL scientists. In the past, the group has done commendable work on the clonal propagation of eucalyptus, teak and bamboo. Nearly a million plants derived from this effort in the TCPP are being grown and evaluated in field trials at more than 30 locations throughout India, in close collaboration with forestry and other experts. The group should now move on to new challenges and goals, and should not be content with the triumphs of the past. The pine project is admittedly difficult and challenging, and requires good understanding of plant physiology, biochemistry and molecular biology. It is a matter of concern, therefore, that the scientific expertise available within the group is rather limited, as almost all have tissue culture training and background. A close collaboration with SLU can thus be most helpful. The group's work on the different species of *Pinus* included in the project at this time can be best described as exploratory. Thus far, no effective methods for large scale clonal propagation have been developed for any of the targeted pine species. Very little quantitative data is

available, and only a small number of plants have been transferred to soil. Exhaustive efforts need to be undertaken immediately. Thousands of cultures must be initiated in order to understand the system. However, on the basis of the limited experiments conducted so far, the development of adventitious buds on cotyledonary explants, and the establishment of embryogenic cultures from immature zygotic embryos, appear to be promising, although both are very low efficiency events at this time. The work on cryopreservation, scale-up in bioreactors, and genetic transformation of pine species is minimal and too preliminary. No formal publications in refereed journals have resulted from the work carried out during the first phase of the project. This is a serious shortcoming.

6. Review of Published Research on Tissue Culture of Pine Species

Regeneration of plants from tissue cultures of a number of species of *Pinus,* including *P. caribaea,* one of the target species at NCL (a summary of the work on this species is provided by David *et al.* 1995), has been described during the past 10-15 years (for detailed descriptions and original references see Jain *et al.* 1995). In most instances regeneration was by somatic embryogenesis, although there are several reports of regeneration by organogenesis and bud proliferation. In a number of instances rooted plants have been grown in soil in greenhouse as well as in the field. Regenerable embryogenic suspension cultures and their successful cryopreservation have been described, as well as transient and stable expression of reporter genes. Review of this literature shows clearly that it should be possible to establish high efficiency regeneration systems for the *Pinus* species targeted by the NCL group, including embryogenic suspension cultures and cryopreservation protocols. Surprisingly and

disappointingly, none of this very significant work, including the very useful work on *P. caribaea* (David *et al.* 1995), which is critically relevant to the success of the SLU/NCL project, is either discussed or cited in any of the project reports or proposals. Much could be learned, and time saved, from a careful study of these publications. It should be noted that the successful work with *Pinus caribaea* (see David *et al.* 1995) was carried out in France, from live material that was shipped weekly by air from Australia.

7. Information Sought in the Terms of Reference

7.1. Relevance and importance. As a developing country with a huge population, India faces a multitude of problems, particularly in the areas of food security, environment, and sustainability. The Government of India has recognised that biotechnology can and should play a major role in the solution of these problems. Therefore, the promotion and application of biotechnology is a national priority in India. The DBT, within the Ministry of Science & Technology, was thus created to spearhead the national initiatives in biotechnology. DBT supports a very wide range of biotechnology projects, including those in the areas of forestry, throughout the country. Although biotechnology R & D in India is slower than expected, it is abundantly clear that biotechnology industry and products will have a major impact on the Indian economy and its citizenry. Successful achievement of the objectives of SLU/NCL project, namely the large scale clonal propagation and improvement of Indian pines, would be of enormous value to Indian forestry, and will directly impact on its economy and social life. It would make a substantial contribution to the national policy of reforestation and sustainable development, and provide a means of livelihood to millions of the poor, particularly women, who rely heavily, if not entirely, on forests for a living. Once established, the

protocols developed in India could be duplicated in other developing countries in the region which have similar problems.

7.2. Impact, sustainability and potential for utilization of the project's results. As stated in 7.1 above, it is clear that the project's results would be of use and value to Indian economy and society. The country clearly has the scientific and technological capacity and competence to continue the R&D work after the termination of the present support from Sweden. There exists in India a large pool of trained scientists who can be instrumental, with appropriate public and private support, to apply the results of the present project to commercial level production of clonal populations of elite trees. In order for such an initiative to succeed, it will be important that the DBT and the Departments of Forestry work closely with scientists and entrepreneurs in establishing and maintaining the necessary production facilities. The experiences of the TCPP at NCL, and other institutions supported by the DBT, can be instrumental in the success of these enterprises.

7.3. The performance of the project. The research carried out at SLU is of high quality, as exemplified by the numerous publications in refereed international journals (see 4). The productivity is also high. Unfortunately, the NCL group has not brought any of its studies to a stage where they can be justifiably published in a quality journal. Consequently, it has no publications to date to its credit. A manuscript on "Gene transfer to embryos of *Pinus roxburghii* and *Pinus contorta*" included in the project report, describes very preliminary results of no major significance. Transient expression of reporter genes is now routine, and by itself is unpublishable in most reputable journals. There are several references to manuscripts under preparation, but no preprints were provided. This is clearly a serious problem, and should be rectified as

soon as possible. Considering the successful work with several pine species, including *P. caribaea* (David et al. 1995, Jain et al., 1995), there is no reason why the NCL group can not establish high efficiency propagation systems for the three selected pine species during the second phase of the project.

As the project is envisioned and structured to be a collaborative research endeavor between the SLU and NCL scientists, it is expected that their efforts will lead to research publications with joint authorship. Sadlly, this very desirable objective has not been achieved, and should be a cause for concern to both SIDA and DBT.

As stated before (see 4), the research program at SLU is of a broad and basic nature. Although it is not specifically targeted to the needs of the research being conducted at NCL, it nevertheless can provide valuable information from its physiological, biochemical and molecular studies of forest species, that can be most useful.

The Swedish and Indian counterparts enjoy a good working relationship, and there are regular annual visits and consultations, but the two parts of the research project can not be considered to form an integrated whole. This should not be taken as a weakness of the project, because of the very different objectives of the research aspirations of the two groups.

7.4. The capacity and competence harnessed to the project. At SLU, no post-docs, graduate students or senior scientists are specifically assigned to work on the SLU/NCL project (only a half-time laboratory assistant is funded by the project). The group is, however, highly competent and represents a broad spectrum of scientific expertise.

The project leader at NCL devotes 50% of her time to the project, and is assisted by one post-doc (50%), three pre-docs (two 100% and one 50%) and two (50% each)

technicians. Most of the NCL scientists have good experience and training in tissue culture techniques. This, however, is also a weakness of the group as it does not provide the breadth of experience - plant physiology, biochemistry, molecular biology - needed to overcome the difficulties of the pine project. Much of the effort and enthusiasm at NCL seems to be directed at the success of the TCPP in the propagation of eucalyptus, teak and bamboo. No sustained and serious effort seems to have been made to develop similar competence with pine species. This must change, and change without much delay. Considerable more effort should be immediately directed toward the challenge of developing equally efficient and successful methods for the large scale clonal propagation of the selected pine species. After all, the processes currently being used in the TCPP are well established, and there is not much new science in the work being performed on eucalyptus, teak and bamboo. It is hard to justify continuing such a routine and well established process at a national laboratory. DBT and NCL should seriously consider transferring the technology developed at NCL to private companies or forestry departments, and use the superb technical facilities and manpower of the TCPP for pine species. A massive, well-planned, well-directed and organized effort must be undertaken immediately if the objectives of the project are to be realized and used for practical benefit to the country. Considerable more scientific effort must be applied to the project.

The scientific equipment acquired through SIDA funding at NCL is in working condition and well maintained. One of the more expensive units is the bioreactor, which is being used for growing the *Picea abies* cell line. Suitable pine cultures are not yet available for growth in bioreactors.

SLU scientists have excellent library and electronic access to contemporary scientific literature. NCL personnel have only limited access, and the documents submitted give the impression that they are not fully aware of the existing literature on the subject. Clearly, more attention needs to be given to literature search and perusal.

The time available to Swedish scientists for research of relevance to the project is satisfactory. On the Indian side, the principal scientist has many administrative responsibilities, including those of the huge TCPP undertaking, and the administration and oversight of the far-flung field trials. She is clearly over-burdened. As suggested above, if the operations of the TCPP in respect to production of planting material of eucalyptus, teak and bamboo, can be shifted elsewhere, then it should be possible for her to devote much more quality time and attention to the pine species. She has earlier demonstrated her ability in tissue culture propagation, and is no doubt upto the task for the pines.

7.5. Poverty alleviation, environmental sustainability and gender equity. It has been stated earlier (see 1) that millions of the very poor in India, particularly women, rely very heavily on forests for their living. Reforestation and conservation of forests would thus be clearly of great benefit to this unfortunate segment of the Indian society. In addition, reforestation and forest conservation would make a substantial contribution to environmental sustainability, a major national policy objective of the Government of India.

It should be noted that the project leaders in Uppsala as well as Pune are women with high scientific qualifications and achievements. Of the seven individuals assigned to the project at Pune, five are women and two men.

7.6. Cost-sharing and cost-effectiveness. Cost sharing between SIDA and DBT appear to be equitable. The proposed budget request from SIDA for SUL (SEK 440,000 each for year 1 and 2, and SEK 460,000 for year 3) is justifiable, and in conformity with the support provided during the first phase of the project.

The requested support from SIDA for project research at NCL needs substantial restructuring. SEK 440,000 is requested for each of the three years. This includes SEK 300,000 each year for equipment. Purchase of equipment in the last year of the project is unjustifiable. It is recommended, therefore, that the total of SEK 900,000 be made available during the first two years. No details of the equipment needed are included in the proposal, but have been provided to me at my request. These funds are to be used to set up a molecular biology laboratory at NCL which will be used for biochemical and molecular analysis of cultures and regenerants to assess their genetic fidelity and uniformity, and for acquiring the necessary equipment for cryopreservation of embryogenic cultures and shoot buds. No more funds should be spent on bioreactors or equipment for genetic transformation. Sufficient travel funds should be provided to cover the visit of one scientist from NCL to SUL each year to learn molecular techniques. The SEK 100,000 annual request for travel is excessive, and should be adjusted to a more reasonable amount.

A molecular biology facility exists at the NCL, but owing to rather serious historical problems and antagonisms it is unlikely to collaborate with the tissue culture group. Forcing the issue will not solve the problem. It will be worthwhile, therefore, to develop the capability within the tissue culture group to carry out biochemical and molecular characterization of cultures and regenerants. Only limited amount of

equipment will have to be acquired to equip the laboratory for such work. Establishing a full-fledged molecular biology laboratory is neither needed nor recommended..

Finally, it does not really make sense to organize an international workshop during the last year of the project. If the workshop is to be held at all - and this needs to be carefully considered and justified in terms of its benefits - it is recommended that it should be held during the second year, so that the project's results can be presented, and it can benefit from interaction and advice of workshop participants. The request of SEK 700,000 for the workshop is totally unrealistic. No more than SEK 400,000 should be provided, and the DBT should share equally in supporting the workshop. Funds provided by the DBT can be used for local expenses of the participants, and travel from neighboring countries. The organization of a successful workshop requires much careful and advance planning. It should begin without delay.

Both SIDA and DBT have provided generous funding for the project. The support provided is appropriate for the type and level of effort needed.

8. Conclusions and Recommendations

8.1. The research effort at SLU is of excellent quality and highly productive. Nevertheless, it is primarily supportive in nature, in the sense that the results obtained there can be usefully adapted for the pine species being worked on at NCL. No changes are recommended in the scope and conduct of this effort.

8.2. The principal objective of the entire project is to develop efficient systems for the clonal propagation of elite trees of selected pine species, so that these can be used in reforestation efforts in India, and possibly other developing countries. The bulk of this work is rightfully being done at NCL as the group there has

extensive experience in plant regeneration from tissue cultures. However, some readjustments in focus and strategy are needed, based on the group's own experience during the first phase of the project, and on collective wisdom available from the published works of many others. In this regard, a number of recommendations are made below.

8.3. All of the work at NCL should be focused on developing clonal systems for the selected pine species. A large number of desirable clones (elite trees) should be selected for clonal propagation, to avoid the problems of monoculture. The group has had some success in inducing adventitious buds on excised cotyledonary explants. Efforts should be made to scale-up the production of such buds via micropropagation in liquid culture on shakers or in large bioreactors (Levin *et al.* 1988, Akita and Takayama 1994, Akita *et al.* 1994). In addition, explants from shoots/plants regenerated in culture should be used to initiate new cultures. They have proved to be very useful in many species.

8.4. The problem of juvenility in plants regenerated from juvenile explants, such as immature embryos or cotyledons, should be kept in mind. Efforts should be made to overcome this by regeneration from mature explants.

8.5. Embryogenic suspension cultures should be developed and grown in simple, disposable, commercially available bioreactors. The sophisticated and rather expensive unit acquired earlier should be used for establishing the culture parameters and requirements. The group at SLU can provide valuable assistance in developing the necessary expertise in maturation and conversion of the somatic embryos.

8.6. The necessary equipment for cryopreservation of embryogenic suspension cultures and shoot buds should be obtained from the funds provided during the first year. In the mean time, the excellent facilities of the National Center for Cell Science at Pune should be used to develop the protocols for the cryopreservation of pine cell cultures. The Center has all the needed equipment, and is willing and capable of providing the necessary technical assistance in this endeavor. Furthermore, the protocols for the cryopreservation of embryogenic cell lines are now well established, even for *Pinus caribaea* (David *et al.* 1995), and widely used. It should not be a major undertaking at all to develop this capability at NCL.

8.7. There are neither any clear objectives nor a pressing immediate need for the genetic transformation of the pine species selected for the project. Transformation should not be done just for the sake of transformation. What traits need to be changed? Do we know enough about the genes that regulate these traits? Are these genes available, and have they been studied enough that they can be used effectively to obtain the desired results? All work on the transformation (also the establishment of synthetic seed systems) of pine species at NCL, which will require considerable effort, experience and manpower, should be abandoned for the time being. Such work can be undertaken in the future, only after efficient regeneration systems have been established, and appropriate genes are available for the manipulation of specific and desired characters. It should be noted that transgenic plants of at least two species of pine have been produced (this work is as yet unpublished), and mapping of the pine genome is being carried out.

8.8. A molecular biology laboratory should be established at NCL to assist in the biochemical and molecular characterization of cell cultures and regenerants.

8.9. SIDA's investment in the project will be well served by having an independent outside expert make annual site visits to the SLU and NCL groups, particularly the latter, to provide strategic technical advice. Many of the problems discovered in the current review could have been avoided had this been done during the first phase of the project. This review should take place just before the annual SIDA/DBT/SLU/NCL meeting, so that its findings and recommendations can be presented and discussed by all the parties concerned.

8.10. The absence of any publications from project research at NCL is a serious matter. Every effort must be made to rectify this problem. It is customary, and often required, that acknowledgment of funding sources be made in publications. No mention of SIDA/DBT funding is made in any of the publications made available to me. This was brought to my attention by officials of one of the funding agencies.

8.11. The focus of effort at the TCPP at NCL should gradually be shifted from eucalyptus, teak and bamboo to the pine species targeted in the project. This will require assignment of additional resources. The goal should be to bring during the next three years the pine project to the same level of sophistication and productivity as the current TCPP project on eucalyptus, teak and bamboo.

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Enclosure A

Terms of Reference for the evaluation of the research project " Tree Tissue Culture"

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1. Sida's approach to research cooperation with India

Given India's strong scientific and technological infrastructure, relative to other developing countries, the **emphasis** that Sida places in research cooperation with India is on producing research **results** rather than on contributing to research capacity building. The **result-emphasizing** research projects should be of high quality by international standards, and should be based on strong **mutual interests** of the Indian and Swedish research institutions. Research cooperation should operate on the principles of professional equality on, and cost-sharing by, both sides. It is expected of the research results that they be of strong relevance not only to India, but also to other developing countries.

2. Background to the " Tree Tissue Culture" research project

A biotechnology research project entitled "Tree Tissue Culture" is ongoing, under the current research cooperation agreement between the Department of Biotechnology (DBT) of the Ministry of Science and Technology, Government of India, New Delhi, and the Department for Research Cooperation (SAREC) of the Swedish International Development Cooperation Agency (Sida), Stockholm.

This project is a joint collaborative research effort between the following two institutions:

* Plant Tissue Culture Division, The National Chemical Laboratory (NCL), Pune, India,

and

* The Department of Forest Genetics, Swedish University of Agricultural Sciences (SUAS), Uppsala, Sweden.

The project coordinators on the Indian and Swedish sides are, respectively, Dr Rajani S. Nadgauda and Prof Sara von Arnold.

The project was launched, in effect, in June 1994, with SAREC and DBT allocating, respectively, a total of SEK 2 million and INR 5.32 million for a three year period.

3. Objectives of the project

The conventional technique of propagating trees vegetatively is by the rooting of cuttings. This method suffers from two significant drawbacks, when it comes to large scale propagation: the cost is too high and the rooting ability declines with increasing age of the mother plant. The biotechnological method of tree tissue culture is designed to overcome these major obstacles. The principal scientific and technological objectives of the project are as follows:

* Clonal multiplication of conifers using micropropagation, organogenesis and embryogenesis;

* Scaling up of embryogenic cell suspension cultures and subsequent growth of somatic embryos in bioreactor;

* Transformation studies to develop transgenic systems; and

* Genetic improvement of Indian conifers by conventional breeding and genetic transformations using gene constructs.

4. The purpose of and the reason for the evaluation

The Indian and Swedish research institutions involved in the project have jointly submitted a draft renewal application to Sida and DBT for continued support for a further three year period (1997-2000). A crucial input into the process of assessing the application for continued support will be an evaluation of the performance of the project so far. In addition, the new application needs to be assessed in the light of the performance so far, as well as on the merits of the proposed future work.

5. The Assignment

Taking due account of the above-mentioned approach by Sida to research cooperation between India and Sweden, as well as the background to and the stated objectives of the project, the consultant shall assess the following:

5.1 Relevance and importance . The relevance and importance criteria have to be assessed in relation, firstly, to the national priorities and policies in India on the role of biotechnology in national development, and, secondly, to the broad interests of economy and society in India. Further, they should also be assessed in relation to the potential usefulness of the project's results to other developing countries.

5.2 Impact, sustainability and potential for utilization of the project's results

These criteria are closely related to, and overlap somewhat, with 5.1 above. What needs to be addressed here is the conjectured impact of the potential practical applications in broad economic and social terms. The assessment of sustainability and potential for utilization involves on the one hand a judgement of whether India has the scientific and technological capacity and competence to continue the R&D work after the Swedish involvement ends, and, on the other hand whether there would be enough interest in India to promote the practical applications.

5.3 The performance of the project

* The consultant shall assess both the quantity and the **scientific quality** of the project's output, in relation to the project's duration so far, where by "output" is meant, firstly, the technology that is being developed, and, secondly, the scientific papers written within the framework of the project.

* With regard to the research papers, it is important to comment on how far the ideal of **joint-authorship** by the Indian and Swedish sides has been realized, and the reasons for significant departure

from this principle of joint-authorship.

* The consultant should look into the question of whether sufficient effort has gone into getting the results published in **refereed** journals of acceptable quality.

* Experience of research cooperation between the Indian and the Swedish counterpart institutions, addressing in particular the following questions: Whether the Indian and the Swedish parts of the project form an integrated whole; the frequency and closeness of the contacts between the Indian and the Swedish scientists; the strengths and weaknesses of the two sides as manifested in their collaborative efforts; and the positive and negative features of the division of labour and the division of roles between the two sides;

5.4 The capacity and competence harnessed to the project

* The overall scientific capacity and competence committed to the project on the Indian and the Swedish sides.

* The actual capacity utilization of the scientific equipment bought for the project with SAREC's funding, their actual working condition and their servicing, repair and maintenance situation;

* The actual availability to the project of essential scientific literature including that bought with SAREC's funding.

* The actual time available to both the Indian and Swedish researchers to devote to the project in relation to the time they have to spend on teaching, administration and other duties.

5.5 Poverty alleviation, environmental sustainabilty and gender equity

* These three objectives, among others, set the framework for and guide Sida's development cooperation efforts. The consultant shall attempt a broad conjectural assessment of the extent to which these three objectives have been taken into account in, firstly, the design and implementation of the project, and, secondly, in the potential impact of the results of project on the supposed beneficiaries.

5.6 Cost-sharing and cost-effectiveness

The consultant shall examine (i) the structure and purpose of the major components of the project budget, and the relative shares that go to the Indian and the Swedish sides, and (ii) the degree of symmetry and balance in the cost-sharing as between the DBT and Sida. On the basis of this examination, the consultant shall assess the appropriateness and adequacy of the budgeted figures for carrying out the research work and other research-related activities

(e.g. travel) presented in the project proposal. Further, he shall assess the cost-effectiveness of the project in comparison with other comparable projects.

6. Conclusions and Recommendations

In addition to submitting his assessment of, and conclusions on, the performance of the project so far, the consultant shall make explicit recommendations on the possible future direction and content of the project, in order to successfully achieve the original principal objectives mentioned earlier on. In this regard, he is requested to critically examine the draft renewal application submitted jointly by the Indian and Swedish sides, paying particular attention to the relevance, importance, scientific quality and feasibility of the proposed future work.

7. Methodology and Time Schedule

7.1 The Methodology
The evaluator will study the published and unpublished written output produced by the project over the period June 1994 - January 1997. He will also examine the project proposal submitted in 1992 on the basis of which the first three-year grant was made, as well as the new proposal submitted in January 1997 for continued support for the three year period 1997-2000. He will inspect the project on site at the Indian and Swedish research institutions and interview the Indian and Swedish researchers involved In the project.

7.2 The Time Schedule
The evaluation will take a maximum of three weeks of work, spread over the period 15 February - 15 April 1997. The consultant will spend the period 15-24 February travelling to and visiting the research institutions in Uppsala and Pune and the DBT in Delhi, in accordance with the itinerary sketched in Enclosure 1. The remaining two weeks will be spent studying the written material and drafting the first and second (final) versions of the evaluation report .

The first draft of the evaluation report shall reach Sida not later than 14 March 1997. This will be circulated by Sida to DBT and the Indian and Swedish project leaders for their comments, which will then be forwarded to the consultant by late March /early April 1997. The second and final version of the evaluation report shall be submitted by the Consultant to Sida not later than two weeks after receiving the comments.

8. Reporting and Publication

The length of the final report shall be **at least** 5000 words (approximately 25 double-spaced typed pages), excluding annexes, but preferably not exceed 8000 words. It should lead with an Executive Summary of not more than 500 words. Further, the evaluators will submit a 400 words summary of the evaluation for publication in Sida's "Evaluation Newsletter" .

The final version of the report shall be submitted in two copies and on disk in WordPerfect or Word for Windows or a compatible format. It should be presented in a form that enables publication without further editing. Subject to decision by Sida, the report will be published and distributed as a publication within the Sida Evaluation Series.

Enclosure 1. The consultant's proposed itinerary

Enclosure 1. The consultant's proposed itinerary

Saturday	Feb 15	DL 955	lv Gainesville	15.15
			ar Atlanta	16.20
		DL 14	lv Atlanta	19.25
Sunday	Feb 16		ar Frankfurt	10.25
		SAS 636	lv Frankfurt	12.35
			ar Stockholm	14.35
		Ground transportation to Uppsala		
Monday	Feb 17		Uppsala	
Tuesday	Feb 18		Uppsala	
Wednesday	Feb 19	DL 2683/SR413	lv Stockholm	08.20
			ar Zurich	10.45
		SR 194	lv Zurich	12.05
Thursday	Feb 20		ar Delhi	00.20
Friday	Feb 21	IA 167	lv Delhi	08.30
			ar Mumbai	10.25
		Ground transportation to Pune		
Saturday	Feb 22		Pune	
Sunday	Feb 23		Pune	
Monday	Feb 24	DL 107	lv Mumbai	01.45
			ar Frankfurt	07.05
		DL 15	lv Frankfurt	11.05
			ar Atlanta	15.15
		DL 1721	lv Atlanta	17.05
			ar Gainesville	18.10

Executive Summary

This is a project jointly supported by Sida and DBT. The project is directed at protein stabilization and use of these proteins in biosensors. This project undertaken by the University of Lund and PROMED has been largely directed at knowledge building, development of techniques and useful protocols, improvements in materials and methods and most importantly, the development of a laboratory with these capabilities that in time could aid in the improvement of the status of the Indian economy and society. The project has been divided between Lund and Madras, with most of the instrumentation developed in Lund and the stabilization chemistries developed in Madras.

DBT has indicated an interest in the development of products from this project. It is unlikely that PROMED can actually develop products. However, they should be able to develop "breadboards" of instruments and protocols for those assays of potential value to industry. The project has been divided into two general areas (1) protein stabilization and (2) biosensor development. The project has undertaken to stabilized β-galactosidase by additives and covalent modification with little success. They have successfully stabilized peroxidase, cholesterol esterase and chymotrypsin. The greatest improvement was observed in the case of cholesterol esterase with the half-life increased by two orders of magnitude.

During the course of this program the grantees have developed an assay for glucose,

aspartame and cholesterol. The assay for glucose used an oxygen electrode and had value only as an educational experience. The aspartame assay has possible value, but this would require conformation by industry. The cholesterol assay, using an enzyme thermistor for readout utilized an heparin-Sepharose column to remove LDL-cholesterol, leaving behind the remainder. In this manner it was possible to determine the following fractions: free cholesterol, total cholesterol, HDL-cholesterol and LDL-cholesterol. The system used a external heparin-Sepharose column, an cholesterol esterase external column and a cholesterol oxidase enzyme thermistor. The assay used organic extraction to obtain the cholesterol from serum samples. Future plans call for elimination of the extraction step and the possible application of chemiluminescence or electrochemistry as the readout, since the cholesterol oxidase produces hydrogen peroxide as one of the products. In addition, PROMED/Lund devised assays for lactate and urea. Present plans call for no further development of any of the enzyme assays other then the cholesterol.

Since Sida has a major interest in environmental issues, the extension of this grant proposes to include the development of a pesticide assay and device utilizing antibodies and fiber optics. I have suggested that they reconsider the possibility of using enzymes because they may not want the specificity of antibodies and by going to enzymes they may be able to develop a platform capable of both the cholesterol assay and the pesticide assay, thus saving a great deal of additional effort that could be used to further develop the one instrument.

The present project produced a significant development, the HDL, LDL, cholesterol assay. The extension proposes to pursue this assay technology to a breadboard stage with the input of industry. It also proposes to develop a pesticide assay system using antibodies. It has been suggested that before initiating either of these projects the grantees contact potential industrial partners and obtain from them specifications that will aid them in deciding on the

readout technologies necessary, the sensitivity, the precision, the accuracy and most of all the acceptable cost of a device to the customer.

1.0 Relevance and Importance of this Project

There seems to be a minor difficulty or misunderstanding regarding the expected objectives of this project. Sida appears to be most interested in the potential this project may have in the areas of bioprocess monitoring and environmental monitoring. DBT, on the other hand, appears to have a greater interest in the biomedical or diagnostic potential of this project. These two interests are not necessarily in opposition. The technology of biosensor and protein stabilization, the two major goals of this project are part and parcel of both bioprocessing and environmental monitoring as well as medical diagnostics. The basic technologies are the same, the milieu is different.

India's national priorities, because of its size, state of industrialization, economy and population is presently more interested in developing inexpensive, rapid, accurate, user friendly diagnostics to aid in improving the health of the Indian population. Sweden, being a further developed country has different priorities such as environmental monitoring, remediation and bioprocess monitoring .

The research project undertaken by the University of Lund and especially PROMED has until now been directed at knowledge building, development of techniques and useful protocols, improvement in materials and methods and most importantly the development of a laboratory with these capabilities that in time could aid in the improvement of the status of the Indian economy and society.

Over the past three years of this project, Dr. Sundaram has been able to build a well equipped working laboratory carrying out applied research in protein stabilization and biosensor technology. He is now ready to put this expertise, experience, and overall technical capability to

the test.

2.0 Impact, Sustainability and Potential for Utilisation of the Projects Results

The project, divided between Sweden and India is also divided by the type of work each side has been carrying out. Lund, has molecular biology capability, electronics capability and easy access to commercially available enzymes. PROMED has no direct access to these materials and capabilities other then through the good offices of the other universities and institutes in the Madras area or other parts of India. This suggests that in the absence of a Swedish input, PROMED will be required to seek these capabilities elsewhere in Madras or India.

There is a large difference between developing a protocol for an instrument and producing a commercial product. In the US, the time required to move a diagnostic or similar product from feasibility to market can be as much as 5-7 years and a great deal more funds than the research originally cost.

Can India sustain and utilize the work that has been completed and will come from PROMED in the future? It will depend a great deal on the input from DBT and industry. For sure, without an industrial input as well, there will be no product output. At best PROMED may be able to produce some type of "Breadboard" instrument for the assays they develop. They probably are in no position to develop a prototype of an instrument for commercialization. Whether DBT will be willing to support a potential product through its development state until PROMED, with much help, or some other organization can produce a true prototype instrument is critical to the final outcome of this project.

When Sida ends its support, it will be up to DBT to guide and support PROMED toward self-sufficiency and DBT's expected goals. These goals, I believe, are product oriented.

Real products do not come from the laboratory. They require a great deal of input from many different sources including: scientists, engineers, manufacturing specialists, and business and marketing people. Even after showing feasibility, e.g. cholesterol detection or glucose detection, the project has only just begun.

In an industrial organization one divides a project into several phases as follows:

1. Basic research: knowledge building.
2. Applied research: once one has an idea, showing that it is feasible, meaning that it works. It does the job.
3. Exploratory development: examine the parameters of the technology, determine the "specifications" for the product. What must it do to be successful, not just technically but in the marketplace as well? This requires input other than technical.
4. Scheduled development: a time table for the development of the product and the product components. Development and application of quality control procedures and finally freezing of the protocols.
5. Manufacturing development: manufacture prototypes, work on the difficulties and problems associated with scale-up. Manufacture and quality control all reagents suitable for clinical trials. Make an actual build of the system for clinical trials. Carry out clinical trials with materials that are "manufactured". Manufacture several lots of reagents and instruments.
6. Manufacturing: make the product on a commercial scale.
7. Marketing and sales: Sell the product and technically support the product.

Based on the above scenario, it is obvious that PROMED can only carry out a project through, at best, the exploratory phase of development. For any technology to become a product, DBT will have to find and provide either through collaboration or licensing to potential manufacturers, direction and possibly additional financial support.

Will India able to take advantage of PROMED's work? At present, I do not feel capable of giving a yes to this question. I can suggest that without sufficient support from Sida and/or DBT, all the efforts up to now, will have been wasted.

Lund has recently indicated that they may be able to give a commercial input from the IDEON companies in Lund. These companies have commercial interests in the sensor field in Sweden. However, even if these Swedish companies move to commercialize the products developed under this grant, a great deal of further assistance will be required from the Indian side. Help for this aspect of commercialization could come from DBT.

3.0 The Performance of the Project

The overall project can be divided into two general areas (1) protein stabilization and (2) biosensor development. The project undertaken during the period of this grant included stabilization of β-galactosidase by both additives and covalent coupling to polymeric carbohydrates. In addition, glutaraldehyde and a polymeric form of the same compound was used for cross-linking. The overall stabilization of this enzyme was disappointing in that no improvement in stability was observed. It is interesting to note that they examined two different forms of the same enzyme. The β-galactosidase from **E. coli** is very large, it is a tetramer and has a molecular weight of approximately 700,000 daltons. The enzyme from **Aspergillus oryzae** is a dimer and has a molecular weight of approximately 150,000 daltons. In addition, this enzyme is a

glycoprotein and contains disulfide bridges, not found in the larger enzyme. However, neither of these enzymes showed any real improvement in stability. Work on this enzyme has ended.

The other major efforts on stabilization was with peroxidase, cholesterol esterase and chymotrypsin. The greatest improvement in stability was observed in the case of cholesterol esterase. The half-life at high temperature was increased by two orders of magnitude. Improvement in both peroxidase and chymotrypsin were observed. However, not to the extent see with the cholesterol esterase.

During his project they have developed biosensor for glucose, aspartame and cholesterol. In all cases they observed values slightly biased but with correlation coefficient in excess of 95% versus a standard assay.

In the case of glucose detection, the method used was one of the first methods described in the literature and probably the poorest choice for determination of glucose. The choice of an oxygen probe for the first electrochemical biosensor attempts were described as a question of availability rather then choice. The group at Lund suggests that the choice of the oxygen probe was for educational value and was not intended to lead to any commercial product. PROMED has indicated that the glucose assay using the oxygen electrode and cyclic voltometry was the work of a student that has sense left the laboratory. The project did demonstrate that a student can develop a biosensor and use biological samples.. They expect to immediately install a potentiostat for modern electrochemical methodologies. The choice of monitoring the decrease in oxygen in an oxygen limiting and oxygen varying system has several disadvantages particularly when monitored amperometrically and kinetically. Since the reaction is dependent on dissolved oxygen, it is possible for the rate to vary not only with the glucose concentration but also with the

oxygen concentration. In addition, the product of the reaction is hydrogen peroxide. This compound is know to denature glucose oxidase. Previous workers monitored the production of hydrogen peroxide directly. This project, as a potential product is most unlikely. Easier and better methods are available that eliminate the oxygen in the equation, and eliminate the production of hydrogen peroxide. This is accomplished by the addition of an electron mediator that shuttles electrons between the enzyme and the electrode surface. This type of system is oxygen insensitive. The only value I find in this project is that it gave PROMED an opportunity to learn something about electrochemistry and apply cyclic voltametry.

The other biosensor studies utilized the enzyme thermistor. By its nature, the enzyme thermistor is difficult to use because it is extremely sensitive to small changes in buffer type, buffer content, flow rates, salt content and a variety of other parameters.

The data generated with this instrument for glucose and aspartame were of high precision and had small standard deviations under laboratory conditions. The data generated for the cholesterol was from normal clinical samples under normal laboratory conditions found in clinical laboratories. Dr. Danielson has indicated that the thermistor has been greatly improved and he should have encouraged PROMED to carry out more clinical analyses with the instrument. The assays for glucose, urea and lactate would have been a good test for the instrument. However, it was outside the scope of the project. However, I still have questions regarding the potential of this device for a clinical laboratory. It has been around for over 20 years with minimal interest. There is no questions that electrochemical techniques also have problems. They are generally not reusable, rather expensive to operate, since the electrodes must be replaced regularly. Continuous systems also have problems related to electrode poisoning, calibration, drift, non-specific

adsorption to membranes decreasing diffusion rates and changing response times and sensitivities. Admittedly, the thermistor and electrochemical techniques both have problems, although most of the problems are different.

A few general comments on the projects:

1. No plans to pursue the β-galactosidase.
2. No plans to pursue the glucose oxidase studies, particularly by monitoring oxygen depletion.
3. Urea determination will be published but not pursued.
4. LDH with the histidine tail is a useful and interesting way of purifying the enzyme. However, no further work is planned here unless there is a need for a biosensor utilizing LDH. The major drawback here is the requirement for a co-factor.
5. Cholesterol, HDL, LDL will be pursued for possible commercialization.
6. Aspartame plans are to continue with an combination of pronase and chymotrypsin as the enzymes of choice. This project needs much more research and industrial input to determine the specifications and need in the food and beverage industries. PROMED has indicated that the project was undertaken after discussions with "diabetologists" in Madras. I suggest that it is the aspartame manufacturer that PROMED should talk with to ascertain the need for an analytical methodology.

Although these projects, at first glance, appear to be independent, closer scrutiny suggests that all

these independent projects, when placed in the context of knowledge building and technology development fit together.

PROMED has concentrated efforts on the chemical modification and stabilization of several enzymes including oxidases, dehydrogenases, esterases and proteases. The methods for stabilization have been consistent, in that PROMED has covalently attached one of several carbohydrate polymers to stabilize the enzymes. In addition, they have examined gludaraldehyde as a cross-linking and stabilizing agent. Overall, one or all of these methods has been successful in improving the stability of the enzymes examined. This stability has been manifested as increased thermal stability, increased urea stability against denaturation, increased lifetime and in some cases improved stability in water miscible organic solvents. All of these characteristics could be valuable for future biosensor. However, I believe that chemical modification as practised by PROMED has limitations that cannot be fully overcome by the methods described in this grant. PROMED feels that chemical stabilization of proteins is a cost-effective method of protein engineering. PROMED also suggests that they are the only group that has undertaken to study protein stabilization by chemical modification in great detail. I will not argue that the approach is less expensive. However, stabilization of a protease in the laboratory is interesting. Can it survive in a wash detergent in a washing machine in the presence of bleach at temperatures between 70 oC to 90 oC? Can PROMED direct the stabilization of a protease to meet specific pre-determined requirements?

Although one can attach many different polymers to a protein, each protein will show different characteristics. The final products will be hydrophilic or hydrophobic, positive, negative or neutrally charged, bind or not bind excess water etc. However, even with these similar

characteristics they will react differently to the stabilizing agent as already seen in this research program. At present, PROMED has no working model on which to base its choice of polymer methods for stabilization. Data is anecdotal and choices of stabilization methods are by "gut feel" or a "shot gun approach". Understanding the genetic and three dimensional structure of thermophilic enzymes and applying this information to gene modification will, I believe, lead to massive improvements in enzyme stability and lead to the addition of desired characteristics of proteins to meet specific needs.

In summary, I believe that the major innovations in protein stabilization will come from molecular biology not chemistry. The present state-of-the-art and the present state of this project are similar. Enzyme immobilization and stabilization is still an art rather then a science.

PROMED has been able to build an arsenal of protocols, techniques, and chemistries that will allow them to move into the next phase of this program, the development of useful protocols that can be further developed into useful products.

There are a few of the protocols already produced in this project that have potential for commercialization. One of these is the cholesterol assay. This assay technology, presently utilizes an enzyme thermistor and is capable of determining not only total and free cholesterol, but HDL and LDL cholesterol as well. At present, this determination in the clinical laboratory is done by calculation. A direct assay would be extremely valuable throughout the world. However, the assay is a long way from being ready for commercialization. It uses pre-treated serum or plasma. No company will accept an assay that requires pre-treatment if there are any other alternatives. Also, the assay uses a thermistor. This instrument is not user friendly and is only a research tool. A different approach must be devised. I have a few suggestions regarding this project. First, find

out what is wanted in the marketplace. What are the specifications required to make this a go? Are there companies willing to work with Lund/PROMED to develop this technology for sale and distribution in India? Industry can provide the information necessary to bring this assay to a point where it can be picked up for development. Replace the thermistor with an different assay vehicle (more later on this).

A second possibility is the aspartame assay. This assay could have value in the food and beverage industries. However, Lund/PROMED have no idea if it is even needed and what the potential user wants or needs. They should immediately contact the proper industrial sources to obtain the necessary answers that will tell them whether to continue or to drop this project. DBT with its connections to the Indian biotechnology industrial community should be able to aid them in making the necessary contacts. Although this assay fits into the food and beverage industries, questions remain, do they already have satisfactory assay methods? Do they require a better assay method? What are the requirements for a better assay? What are the requirements for stability, throughput, cost, etc. Only industry can answer these questions.

3.1 Publications

There have been ten papers written. Five of these are jointly between Lund and PROMED. The remaining five are be members of either side but relate to the project. Of these ten papers, most are submitted, in press or in progress. Both sides admit that they have been lax in moving these papers along. A couple of these papers have been in progress for 3 years. There is a great deal of room for improvement. Several of the papers were the result of posters at various conferences and were published in conference proceedings. At present, only two of the papers have been published in peer reviewed journals and the remainder are either in the review

process or still sitting on someone's desk in Lund or Madras. More effort and interest is required on both sides. On of the major difficulties is distance. Communication has been a problem since PROMED has no e-mail and FAX messages sometimes do not get through. Improvement in communications should create a major improvement in the publication record in the future.

3.2 The Relationship

Both Sundaram and Danielson have an excellent working relationship. Each has visited the other several times over the course of the program. In addition they talk weekly over the telephone. The relationship appears to work well, with each side performing their tasks best suited to that side. Because this project is only one of many in Sweden, with Dr. Danielson is only putting 5% of his time on the project in Lund, although, in addition to the time in Lund, he has spent a great deal of his time in Madras. His efforts and his concerns for the project are not always foremost in his mind. Dr. Sundaram however, spends, at least, 60% of his time on the project making it his most important program.

The scientific capacity of Lund far exceeds that of PROMED. The effort on the Swedish side has been far less than at PROMED. In addition to Danielsons 5%, as previously mentioned, there is the time Danielson has spent in Madras. Totally he believes that an accurate figure would be approximately 25% of his total time spent on this project. In addition, a technician spends approximately 50% on the project. At PROMED, in addition to Dr. Sundaram, he has had several persons working on the project utilizing anywhere from 20% to 60% of that persons time. The staffing has been as follows: Sundaram (Director) 60%, Venkatesh, 50-60%, Sivakumor, 40%, Rajalakshimi 20%, and Shubhada 40%. Several of these persons are no longer at PROMED and are therefore no longer working on this project.

4.0 The Capacity and Competence Harnessed to the Project

The overall scientific competence on both sides has been excellent. The commitment on the Indian side has been, as expected, greater then that of Sweden.

The lab at PROMED has been well equipped and the equipment has been well utilized in pursuit of this project. Repair and parts are always a problem in India and some of the equipment is out of order due to lack of parts. However, PROMED appears to keep their equipment, generally in good condition and in working order.

PROMED subscribes to a few journals. Not really enough to truly keep abreast of the literature. A few major journals are lacking, particularly as they relate to biosensor technology. Additional funds for journals would be valuable. Most of all, PROMED needs E-mail and access to the Internet worldwide web.

5.0 Cost-Sharing and Cost Effectiveness

A portion of the work undertaken at Lund was not paid for by the Sida grant. The development of the enzyme thermistor was paid from other sources, the recombinant programs developed at Lund were paid by other sources. It is obvious that since Lund started with an existing laboratory and facilities, they have been in a position to contribute more in-kind services then has PROMED.

The budget for PROMED was sufficient to help purchase necessary equipment and to obtain technical support. Dr. Sundaram's salary (as I understand) did not come from this grant and was therefore in-kind services. This is also true of facilities and some of the equipment. The travel budget did permit some exchange of personal. Dr. Danielson visited Madras over the last three years approximately 6 times, while Dr. Sundaram visited Lund 4 times. I believe that

exchanges are one of the more important aspects of this program and should be expanded to include more persons for longer times. I would like to see more funds for more exchanges between the two sides for both short visits (2-3 weeks) and longer visits of several months. These visits will expose scientists, particularly Indian scientists and students to a different culture, a broader technology base and new opportunities to learn. In addition, they may initiate new research projects with scientists from the other side that could continue upon their return home. The more interaction, the better.

6.0 Conclusions and Recommendations

I have some immediate concerns regarding the choice of enzymes for stabilization. Although it is true that PROMED/Lund were trying to develop a "generic" approach to stabilization, it appears to me to be more of a shotgun approach. The choice of enzymes should not have been based on availability and cost, but on how and where they could be used and why. In addition, I would have much like to see more studies with organic solvents carried out using water immiscible as well as water miscible solvents as was performed in the case of cholesterol. If one reads the present literature, it is obvious that almost all the organic solvent work is in immiscible solvents and generally devoted to organic synthesis. Biosensor capable of monitoring bioprocesses in organic systems (water immiscible) would have value to the product manufacturer. I suggest that if stabilization studies continue they should include more work on water immiscible solvent systems, particularly with the cholesterol and pesticides.

Examination of the PROMED report on this project submitted in January 97 is written in such a way as to give the suggestion that the projects do not appear to tie together. However, on more careful examination, it is obvious that PROMED chose to attempt to stabilize "available"

enzymes by the attachment of one of 3 or 4 polymeric carbohydrates or to cross-link the enzymes with a cross-linking agent. The hope, of course, was that the above agents would be sufficient to develop a generic approach to stabilization of any enzyme. I do not believe that this has been the case. Although I have my doubts as to the future of chemical modification, PROMED could have taken a more systematic approach to the problem. PROMED has indicated that the agents were all directed at binding to the ϵ-amino group of lysine. I have several questions here.

1. What is the effect on each type of enzyme by the modification of specific numbers of amine groups?
2. What about modifying the other available functional groups including carboxyl groups, hydroxyl groups, phenolic groups, sulfhydryl groups etc?
3. What about modification of the carbohydrate portion of glycoproteins?
4. What happens to lipoproteins when modified?
5. What about cross-linking agents that react with other functional groups?

Since the approach was not systematic, no model has been developed and no real predictions can be made regarding stabilization. The other major endeavor was the development of a "biosensor". Here the project examined two approaches, electrochemistry and the enzyme thermistor. The electrochemistry project, although yielding limited information did acquaint the group with the methodology. The enzyme thermistor that was provided to PROMED by Lund yielded excellent data and indeed showed that it is possible to monitor aspartame, urea, and cholesterol in it several forms. This aspect of the project was most successful. The cholesterol assay that includes HDL and LDL-cholesterol is important and possibly valuable and well worth continuation. The aspartame project is worth following only if there is an industrial need in India

for an instrumented method. In all cases, the instrument should not be the thermistor. However, I have been informed that major advances have been made in this instrument and that my information may not be up to date. So far, this instrument has not been used in a commercial environment. I strongly suggest devising a different instrument system for further development. PROMED is aware of this concern and is in agreement. In the case of the cholesterol assay or any other assay that may produce hydrogen peroxide, electrochemistry or possibly chemiluminescence may be preferable. Since cholesterol oxidase produces hydrogen peroxide, it can be detected electrochemically or by the addition of luminol or isoluminol to produce luminescence. I suggest that they use the enzyme thermistor only as a research tool and develop the assays with a different technology. I will admit that if Lund and PROMED can justify further development with the enzyme thermistor, I would not be totally adverse to their decision.

In summary, the previous 5 to 7 years have been well spent in building a lab at PROMED, training students and scientists, developing methods and protocols useful in future programs and finding a niche in the biotechnology research establishment in India. The grant has produced a cholesterol assay that could become commercial and an aspartame assay that may be of value.

7.0 Extension Proposal to Sida and DBT

The extension focuses on two major topics: (1) The development of a cholesterol assay for commercialization in India. (2) Development of (fiber)fluorimetric/luminmetric technique and device for detection of pesticides.

The decision to divide the proposed extension into two major projects, the first clinical in nature and the second dealing with environmental issues is related to the major interests of the funding agencies. DBT is most interested in improving the health of the Indian population

through applications of biotechnology. Sida is most interested in improving the environment of Sweden a nation with quality health care already in place.

The cholesterol assay that has been developed by PROMED/Lund can distinguish between HDL- and LDL-cholesterol and measure total and free cholesterol. This assay would be of great value, not only in India but in other parts of the world including Sweden. The assay has been carried out using an enzyme thermistor provided to PROMED by Lund. The device is loaded with cholesterol oxidase/catalase with heparin-Sepharose and cholesterol esterase in separate columns outside the thermistor. The heparin-Sepharose separates HDL- from LDL-cholesterol while the cholesterol esterase hydrolyses the cholesterol esters to cholesterol. The samples require organic solvent extraction.

The development of the fiber optic based pesticide sensor is based on the experience at Lund with fiber optic immunoassay and some field applications of these immunosensors. In addition Lund has capabilities for the production of monoclonal and polyclonal antibodies.

7.1 Major Goals and Issues Regarding Cholesterol Analysis

The development of a breadboard device for cholesterol assays including some form of sample pre-treatment is the present situation using the thermistor. Direct assay of serum, blood or plasma without any extraction is the goal and the challenge. The proposers plan to examine additional technologies for detection and quantitation of cholesterol including fluorimetry, luminescence and amperometry. The hope is that one of these methods will work successfully without the need for organic extraction of the sample. The enzyme thermistor will be used as a reference instrument for comparison with the other technologies planned for examination. Since hydrogen peroxide is the major product of the cholesterol oxidase reaction, it is an excellent

candidate for product detection. Chemiluminescence and amperometry are likely candidates for this type of product.

As part of the project, PROMED plans to continue working on stabilization of the required enzymes. Good results, previously obtained with the cholesterol esterase are the impetus for this decision. How important it will be to stabilize the cholesterol oxidase is problematical. There is extensive literature on this enzyme for cholesterol oxidation and its application to cholesterol analysis. Surely, there is information on the stability of the natural enzyme. If it is not necessary to stabilize this enzyme, it would be wasteful of time and money to proceed with this aspect of the project when not necessary.

7.2 Major Goals and Issues Regarding the Pesticide Analysis

Lund has experience and expertise in fiber optics and optrode technology for immunoassay. Screen printed slides containing antibodies, chemical indicators and possibly competitive labeled antigens are possible devices for pesticide analysis. They plan to use a CCD camera for detection. Capillary tubes filled with these reagents will be considered as a means of further miniaturization. Both bioluminescence and chemiluminescence are being considered as readout methods. Lund has the capability of producing the necessary monoclonal and polyclonal antibodies for an assay device. They also have the capability for protein engineering, phage library display and are willing to transfer and train PROMED personnel as required.

7.3 Research Tasks

Tasks listed for the cholesterol diagnostic appear to be reasonable, but lacking in detail (lack of space?). I am not sure why they wish to examine all these different readout methods. Lund indicated in previous conversations that the enzyme thermistor is much more reliable and

easier to use then previously. If true, why not continue with this instrument? I suggest that they should obtain the necessary specifications for their potential customers and then try to make some rational decision as to technologies.

The suggestion that they start this project with an initial input from industry is excellent. If fact, industry may suggest to them, which technologies they would most prefer to use in their facilities. Industry should be able to give PROMED/Lund specifications for cost per test, time per test, sensitivity required, precision necessary, accuracy requirements and even whether they want continuous or single individual assays using some form of disposable cartridge or card.

Fiberfluorimetric analysis for pesticides is planned as an immunoassay. Why did the choose an immunoassay rather then an enzyme assay? There are several enzymes that are quite specific for classes of pesticides e.g. carbamate's, organophosphate's, etc. Do they really want or need the specificity obtained by use of immunoassay. It is true that immunoassay should be far more sensitive. This could be a reason. Industry would be very useful here. I suggest they talk with industrial people at the outset before initiating an immunoassay methodology. It is true that an enzymatic approach will be less generic. It will not be extendable to hormones and other clinical analytes. However, it would potentially allow for both types of assays on the same platform and it is not a question of what PROMED/Lund find interesting but what the customer wants.

7.4 General Comments on Proposal Extension

Overall, the proposal is excellent. They have narrowed their interests to two major projects. They may wish to narrow their choice of technologies as well, once they have discussed industries needs, desires and specifications for the products they are planning on developing. I

suggest that PROMED/Lund touch base with several industrial groups before actually starting work and finding themselves committed to a technology and an architecture that will not meet the customers needs.

The extension proposes increased interactions between laboratories with increased visits and research exchanges between Lund and Madras. This expansion is important and should be supported by both Sida and DBT. In addition, it is very important for Madras to obtain e-mail facilities inorder to facilitate discussions and increase the rate and timing of publications co-authored by group members.

The idea of yearly mini-symposia to increase contacts with other institutions and industries in India is an excellent idea. I would emphasis the industrial contacts that could lead to collaborations and possibly additional financial support for PROMED. These meetings should be kept small and companies invited should have some interest in either the technology for other possible applications or the analytes that will be under discussion at the symposium. The idea of a international meeting is very nice if special funding is made available. However, the cost of even a relatively small meeting is in the range of $50,000 to $70,000. The value of an international meeting is not as great nor is it as important as the smaller yearly symposia. It will be useful for increasing PROMED's visability, but less important in finding potential Indian industrial partners.

DBT is interested in "deliverables". This extension proposal is designed to deliver two devices, at least at the breadboard level. The devices are for cholesteral and pesticide determination. It would be useful, but not required, if both these assays could be performed on the same platform. PROMED/Lund may wish to reconsider enzymes versus antibodies for the pesticide analysis.

In summary, this is an excellent extension of the previous program and deserves funding by Sida/DBT. It satisfies both the environmental and clinical desires of the funding agencies. If successful, this program should lead to the development of commercial products of interest to industrial partners.

Sida

Department for Research
Cooperation, SAREC
M R Bhagavan

1997-02-24

Enclosure A

Draft Terms of Reference for the evaluation of the research project " Natural and Recombinant Proteins/Enzymes for Use as Biosenors"

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1. Sida's approach to research cooperation with India

Given India's strong scientific and technological infrastructure, relative to other developing countries, the **emphasis** that Sida places in research cooperation with India is on producing research **results** rather than on contributing to research capacity building. The **result-emphasizing** research projects should be of high quality by international standards, and should be based on strong **mutual interests** of the Indian and Swedish research institutions. Research cooperation should operate on the principles of professional equality on, and cost-sharing by, both sides. It is expected of the research results that they be of strong relevance not only to India, but also to other developing countries.

2. Background to the evaluated research project

A biotechnology research project entitled " Natural and Recombinant Proteins/Enzymes for Use as Biosenors " is ongoing, under the current research cooperation agreement between the Department of Biotechnology (DBT) of the Ministry of Science and Technology, Government of India, New Delhi, and the Department for Research Cooperation (SAREC) of the Swedish International Development Cooperation Agency (Sida), Stockholm.

This project is a joint collaborative research effort between the following two institutions:

* Centre for Protein Engineering, Madras, India,

and

* The Department of Pure and Applied Biochemistry, University of Lund, Sweden.

The project coordinators on the Indian and Swedish sides are, respectively, Prof P V Sundaram and Dr Bengt Danielsson.

The project was launched, in effect, in the autumn of 1993, with SAREC and DBT allocating, respectively, a total of SEK 1.8 million and INR 0.904 million for a three year period.

3. Objectives of the project

The major purpose is to develop generic techniques using proteins and enzymes as biosensors for a range of applications in industry, medicine and environmental rehabilitation. The focus is on ELISA tests, metal detecting biosensors and enzyme thermistors, which constitute advances over existing methods. The principal scientific and technological objectives of the project are as follows:

* Production of recombinant cells containing proteins of specific, modified structure.

* Development of polymers for making columns or immobilized proteins of various physical characterizations, using the above-mentioned recombinant cells.

* Biosensor development, and demonstration of their use in analysis.

4. The purpose of and the reason for the evaluation

The Indian and Swedish research institutions involved in the project have jointly submitted a draft renewal application to Sida and DBT for continued support for a further three year period (1997-2000). A crucial input into the process of assessing the application for continued support will be an evaluation of the performance of the project so far. In addition, the new application needs to be assessed in the light of the performance so far, as well as on the merits of the proposed future work.

5. The Assignment

Taking due account of the above-mentioned approach by Sida to research cooperation between India and Sweden, as well as the background to and the stated objectives of the project, the consultant shall assess the following:

5.1 Relevance and importance . The relevance and importance criteria have to be assessed in relation, firstly, to the national priorities and policies in India on the role of biotechnology in national development, and, secondly, to the broad interests of economy and society in India. Further, they should also be assessed in relation to the potential usefulness of the project's results to other developing countries.

5.2 Impact, sustainability and potential for utilization of the project's results

These criteria are closely related to, and overlap somewhat, with 5.1 above. What needs to be addressed here is the conjectured impact of the potential practical applications in broad economic and social terms. The assessment of sustainability and potential for utilization involves on the one hand a judgement of whether India has the scientific and technological capacity and competence to continue the R&D work after the Swedish involvement ends, and, on the other hand whether there would be enough interest in India to promote the practical applications.

5.3 The performance of the project

* The consultant shall assess both the quantity and the **scientific quality** of the project's output, in relation to the project's duration so far, where by "output" is meant, firstly, the technology that is being developed, and, secondly, the scientific papers written within the framework of the project.

* With regard to the research papers, it is important to comment on how far the ideal of **joint-authorship** by the Indian and Swedish sides has been realized, and the reasons for significant departure

from this principle of joint-authorship.

* The consultant should look into the question of whether sufficient effort has gone into getting the results published in **refereed** journals of acceptable quality.

* Experience of research cooperation between the Indian and the Swedish counterpart institutions, addressing in particular the following questions: Whether the Indian and the Swedish parts of the project form an integrated whole; the frequency and closeness of the contacts between the Indian and the Swedish scientists; the strengths and weaknesses of the two sides as manifested in their collaborative efforts; and the positive and negative features of the division of labour and the division of roles between the two sides;

5.4 The capacity and competence harnessed to the project

* The overall scientific capacity and competence committed to the project on the Indian and the Swedish sides.

* The actual capacity utilization of the scientific equipment bought for the project with SAREC's funding, their actual working condition and their servicing, repair and maintenance situation;

* The actual availability to the project of essential scientific literature including that bought with SAREC's funding.

* The actual time available to both the Indian and Swedish researchers to devote to the project in relation to the time they have to spend on teaching, administration and other duties.

5.5 Poverty alleviation, environmental sustainabilty and gender equity

* These three objectives, among others, set the framework for and guide Sida's development cooperation efforts. The consultant shall attempt a broad conjectural assessment of the extent to which these three objectives have been taken into account in, firstly, the design and implementation of the project, and, secondly, in the potential impact of the results of project on the supposed beneficiaries.

5.6 Cost-sharing and cost-effectiveness

The consultant shall examine (i) the structure and purpose of the major components of the project budget, and the relative shares that go to the Indian and the Swedish sides, and (ii) the degree of symmetry and balance in the cost-sharing as between the DBT and Sida. On the basis of this examination, the consultant shall assess the appropriateness and adequacy of the budgeted figures for carrying out the research work and other research-related activities

(e.g. travel) presented in the project proposal. Further, he shall assess the cost-effectiveness of the project in comparison with other comparable projects.

6. Conclusions and Recommendations

In addition to submitting his assessment of, and conclusions on, the performance of the project so far, the consultant shall make explicit recommendations on the possible future direction and content of the project, in order to successfully achieve the original principal objectives mentioned earlier on. In this regard, he is requested to critically examine the draft renewal application submitted jointly by the Indian and Swedish sides, paying particular attention to the relevance, importance, scientific quality and feasibility of the proposed future work.

7. Methodology and Time Schedule

7.1 The Methodology

The evaluator will study the published and unpublished written output produced by the project over the period June 1994 - January 1997. He will also examine the project proposal submitted in 1992 on the basis of which the first three-year grant was made, as well as the new proposal submitted in January 1997 for continued support for the three year period 1997-2000. He will inspect the project on site at the Indian and Swedish research institutions and interview the Indian and Swedish researchers involved In the project.

7.2 The Time Schedule

The evaluation will take a maximum of three weeks of work, spread over the period 10 March - 30 April 1997. The consultant will spend the period 10-19 March travelling to and visiting the research institutions in Lund and Madras and the DBT in Delhi, in accordance with the itinerary sketched in Enclosure 1. The remaining time (about 12 days) will be spent studying the written material and drafting the first and second (final) versions of the evaluation report .

The first draft of the evaluation report shall reach Sida not later than 4 April 97. This will be circulated by Sida to DBT and the Indian and Swedish project leaders for their comments, which will then be forwarded to the consultant by about mid April 1997. The second and final version of the evaluation report shall be submitted by the Consultant to Sida not later than two weeks after receiving the comments.

8. Reporting and Publication

The length of the final report shall be **at least** 5000 words (approximately 25 double-spaced typed pages), excluding annexes, but preferably not exceed 8000 words. It should lead with an Executive Summary of not more than 500 words. Further, the evaluators will submit a 400 words summary of the evaluation for publication in Sida's "Evaluation Newsletter" .

The final version of the report shall be submitted in two copies and on disk in WordPerfect or Word for Windows or a compatible format. It should be presented in a form that enables publication without further editing. Subject to decision by Sida, the report will be published and distributed as a publication within the Sida Evaluation Series.

Enclosure 1: The consultant's proposed itinerary

Enclosure 1. The consultant's proposed itinerary

Monday, 10 March : Flight Washington-Amsterdam-Copenhagen

Tuesday, 11 March: Arrival Copenhagen and travel to Lund

Wednesday, 12 March: Visiting the Dept of Pure and Applied Biochemistry at Lund University, including a meeting with Dr Bhagavan of Sida.

Thursday, 13 March: Flight Copenhagen-Frankfurt-Delhi

Friday, 14 March: Forenoon: Visiting DBT in New Delhi
Evening flight to Madras

Saturday 15 March and Sunday 16 March in Madras: Further studying of the project's output and commencement of the first draft of the evaluation report

Monday 17 March and Tuesday 18 March in Madras: Visiting the Centre for Protein Engineering.

Wednesday 19 march: Flight Madras-Delhi-Frankfurt-Washington

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