REPORT OF THE EVALUATION

OF THE BIOTA-FAPESP PROGRAM

BIOprospecTA

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Introduction

This report is based on the reading of the descriptions of the output of the various projects in the program, the presentations during the symposium, the discussions with the scientists at the poster sessions, the self evaluation presented by the program coordinator and the 2009 Science plan and strategy as proposed by the BIOTA program. As prof. dr. J. Gloer did not receive his visa in time, this assessment was made by one committee member only. But extensive discussions with the other three members of the committee that assessed the BIOTA project resulted in a clear picture about the coherence of the programs. I wish to express my sincere thanks to all the hosts, the committee members and the project coordinators and researchers for the open and frank discussions.

The results from the past years as presented during the evaluation meeting by the BIOprospecTA project clearly show that the various vectors represented by the participating research groups are getting aligned and are pointing in the same direction. Obviously there are many interactions and a steady flow of interesting results is coming out, resulting in excellent publications in the leading international peer reviewed scientific journals. Also internationally the project is getting quite some attention as can be learned from the number of plenary presentations that members of the consortium have given at international scientific meetings. In the book series "Comprehensive Natural Products Chemistry II, Elsevier, Oxford, 2010" the project is given as an important international example of how a bioprospecting project can be organized and what results has been achieved. Also the journal Science reported on the unique BIOTA project.

Besides the scientific output there is also an excellent academic output in terms of MSC- and PhD-students. This results in a new strong generation of natural products researchers. This with the already well internationally recognized existing high level of natural products research is an important resource of the Sao Paulo state and of the country, which in combination with the extremely rich biodiversity and thus of chemodiversity, has a great economic potential. This sleeping giant is waiting for being waken up by the government by facilitating the exploration of biodiversity to the benefit of the local, regional, national and international society.

From the reports it is clear that in the present situation the exploration of biodiversity is seriously hampered by a very slow licensing system. With a biodiversity that does not know any political borders, there is a considerable risk that all the good bits in the meantime will be discovered in neighboring countries. To come to an economic exploitation a country like Finland may serve as an example, with only a few hundred of native plant species they have given the world compounds like xylitol and stigmastenol, which gave the Finnish industry novel products to produce. In that model any interesting compound obtained from existing agricultural processing is made freely available to anyone to test. Any application will result in revenues for the producer of the compounds. In the context of the present project one may envisage that libraries of isolated compounds and plant extracts are made available (for a small fee?) to pharmaceutical, cosmetics and agrochemical companies for screening for activities. By negotiating contracts with companies the potential revenues can be secured. Such a model of exploitation could be organized in the sidelines of the project, as it is not really an academic function, a (spin-off) company or a valorization office from the FAPESP would be more suited for such a task.

There are contradictory interests in the academic research and the patenting of promising innovative results. To profit most of a patent one should develop the process or product as much as possible before patenting, as every year that a patent covers the commercial phase of an innovation gives extra revenues. But that means that results from MSc- or PhD-theses might have to be kept confidential for prolonged periods. In fact universities are not made for developing products, but their primary task is to develop science

that may give rise to ideas, and their major output is students with MSc and PhD diplomas. These students could be the ones that make products from ideas, which however means that they should have the chance to be trained in biobased business. That could be the basis for spin-off companies taking care of the valorization of the research.

The international scene, novel opportunities and challenges

In this changing world there are a lot of opportunities for bioprospecting. Drug leadfinding in big pharma is being cut enormously, consequently there is an opportunity for new companies that generate new compounds, hits and/or leads, e.g. by screening biodiversity and further develop the natural products leads via medicinal chemistry. On any level of this development one may commercialize the findings, but this requires an active policy for commercialization from FAPESP or the program.

Another important trend is the development of traditional knowledge about food and medicine in Asia, where billions of dollars are invested by the local governments in coming to evidence based traditional medicines or developing novel drugs based on this. A systems biology approach is now the trend, as it is anticipated that many traditional medicines will have multiple actions, including synergy, which cannot be dealt with in the reductionist approach of bioassay-guided fractionation which is now the approach in pharmaceutical industry and most bioprospecting programs. Also for Brazil such a novel approach seems of interest. First steps have already been made by implementation of metabolomics technology.

To further develop this in the context of BIOprospecTA, novel bioassays on the level of cells or whole organisms are required, which mean closer collaboration with pharmacology. It is important to further develop the hits now produced in the bioprospecting projects into real leads. Novel test systems like zebrafish and transgenic cell lines and animals could play an important role in this, but this requires developing novel collaborations with pharmacology and toxicology.

The third major trend is connected with the urgent need for novel antibiotics. Multidrug resistant microorganisms are rapidly spreading and caused already many casualties. The biodiversity mapped in the BIOTA program, including many plants and microorganism from quite unique sources, offers ample opportunities for screening for antibiotics. As big pharma has little interest in this field this is typically an interesting opportunity for Brazil.

Bioprospecting is more than finding novel biologically active compounds, it is also about new concepts, new proteins/enzymes, new genes, new food, new fibers. Our ancestors find all the plants for food, medicine, clothing, shelter and fuel in their direct environment by thorough observations, but without the help of all scientific equipment we have now. An interesting example presented during the meeting is a specific bee species as pollinator for strawberry flowers that makes that the strawberry gets a better shape. Thus ecological observations may lead to novel concepts with commercial potential.

Microorganisms in itself are also a useful resource when libraries are available for screening for all kind of different properties, such as the presence of antibiotics, biotransformations, plant growth or resistance enhancing activity, biosynthetic genes for novel compounds like polyketide synthases encoding genes for recombinatorial biochemistry, etc. Such libraries of microorganisms do have commercial value in itself. So building up libraries of organisms, extracts, compounds, enzymes and genes will be important for making a platform for bioprospecting. The upcoming novel microbiology projects should play an important role in creating such a platform for the microorganisms.

So besides bioprospecting based on at random screening of large numbers of samples one could first make an intelligent virtual screen of the libraries and databases to choose organisms to study for certain objectives, e.g. based on ecology, ethnobotany or exploring the

virtual biological and/or chemical space. The studies on phylogenetics (see also BIOTA assessment report) are an important step in the direction of combining BIOTA data with identifying chemodiversity hotspots for bioprospecting. The present and future BIOprospecTA projects should have their roots in BIOTA, and develop tools for efficient datamining in the BIOTA database.

The BIOTA database sinBIOTA is an excellent platform for developing different lines of bioprospecting, thus giving extra value to the biodiversity. In certain cases this will mean that a sustainable production of the organism is required, in other cases it may result in (semi)synthetic products or biotechnological production. By better understanding the ecological systems, one will find new leads for sustainable agri- and horticulture and at the same time better understand the needs of conservation of all species to keep biodiversity, e.g. no pollinator, no seed, no plant!

Present situation BIOprospecTA

With the overview given above, the question is if the BIOprospecTA program is ready for the challenges to make use of the new global opportunities and the needs of the society. The September 2009 "BIOTA Science Plan and Strategies for the next Decade" has a clear analysis of the role of the BIOprospecTA project and already pointed out that the pharmacology and toxicology components of the project need to be strengthened. Also the need of a proper database is mentioned. Such a database for the results obtained in the project, both chemically and in terms of biological activity, should support fast dereplication of active extracts.

In the self-evaluation presented by the group during the meeting the importance of finalizing the design of this database and linking it to sinBIOTA was mentioned as a high priority. Also the broadening of the project with groups contributing to studies of the activities was seen as an important item. In terms of external threats the major concern was the great difficulty in getting licenses for bioprospecting. Maybe the offices concerned should become faciltitators of bioprospecting instead of law enforcement inspectors. In terms of opportunities collaboration with industry comes out as an important item, particularly concerning the types of bioactivities industry would be interested in. Moreover, potential collaboration with industry for screening extracts and compounds needs to be considered.

Considering the project descriptions and the reports on the output, the strength and weaknesses will be discussed below. But first the actions on recommendations of the last report will be considered.

Recommendations 2009

1. Scheme of the total BIOprospecTA network including names of coordinators and project leaders is not yet available. Such a scheme would in fact be useful for the website. The clear workflow presented for the "Platform for in-vivo and in-vitro metabolism studies" could serve as an example.
2. Internationalization: the Science paper and the Comprehensive Natural Products Chemistry II paper put the project on the map, but the website needs to be updated and extended, also to advertise the bioprospecting efforts to potentially interested academic or industrial partners.
3. Database has high priority but is not yet ready, so it is difficult to judge the value. Also the coupling to the sinBIOTA database is obviously a need, but has not yet been achieved as the sinBIOTA 2.0 is still in the prototype phase. This recommendation thus remains.
4. Patents: considering the output not much patents have been applied for in the past period. This might be due to the lack of a proper protocol to follow for the researchers

when some innovative results are obtained. This point thus remains on the list of recommendations and should be considered as a high priority as it is the basis for valorization and for new business.

5. Commercialization extract and compound library: in fact the same situation occurs here as for patents, the problem might be that different universities are involved, making valorization difficult. As suggested above, one might consider to either start a central valorization office or to hire an external company to take care of this.
6. Develop model for valorization through workshop with local/regional companies to discuss mutual interests. Like for the previous recommendation action still needs to be taken.
7. Increase expertise in pharmacology, toxicology and molecular biology in the project. This is high priority but no real results yet, also in this case a workshop could be useful to make an inventory about possibilities. This in connection with the workshop with potential industrial partners could give a clear direction how to go forward.
8. Prioritizing compounds for further study, see previous recommendation.
9. Novel projects in the framework of BIOprospecTA, it seems that a large number of projects in the field of microbiology will be funded, but at present their role in connection with BIOTA and/or BIOprospecTA objectives is not known yet.
10. Internationalization, and use of English. The meeting was this time mostly in English, so clearly progress has been made in this sense.
11. In conclusion, most of the previous recommendation have been followed up, though not in all cases the final goal has been achieved.

Strong points of the program

1. The group presented in a clear and efficient way the projects and the major achievements and problems, showing that they are functioning quite well as an organization.
2. The BIOprospecTA group consists of world class natural products chemists with ample experience in the field of isolation and structure elucidation of natural products. All work with the same objective finding novel biological active compounds. The project has been able to get all “vectors” pointing into the same direction.
3. The program has an outstanding output in terms of publications, though there is a difference between the groups, which can be explained in most cases by the quite different size of the projects.
4. The project groups are publishing in the top journals for the field. They have given presentation as invited lecturers at a number of international meetings.
5. Strong educational program for a new generation of scientists that can contribute to the exploration and exploitation of all of the Brazilian biodiversity.
6. There is a good infrastructure for the research and FAPESP has programs to help financing big equipment like NMR facilities. Good collaboration between groups will allow an efficient use of such facilities, i.e. 24 hrs per day, 365 days per year.
7. A start is made to add chemical information to the BIOTA data, the phylogeny based on chemistry will be an important tool for structuring the BIOTA data and thus identify chemodiversity hotspots.

Weak points

1. Though sharing a common objective there might be more interaction between the groups. For example now it is difficult to see the interaction between the projects, e.g. what is the number of joint publications between 2, 3, or more groups. A preliminary search for this showed in fact that the number of joint papers is limited and some

groups are more interactive then others, probably also due to the theme studied. A scheme showing the interactions and common activities would be useful.

2. Certain activities should be organized like a central platform, e.g. for the screening of biological activity. To appoint one person in charge of this would help to enable a systematic screening of all compounds and extracts by setting up standard operation protocols (SOPs) for e.g. extraction methods, dose levels tested, and setting standards for what should be considered an active dose that should lead to further studies. Also a strategy to identify in an early phase compounds that are toxic needs to be adapted to avoid loosing precious time that could be better used for the development of real promising lead compounds. The wish expressed by the project leaders to increase collaboration with pharmacology, toxicology and molecular biology also asks for a responsible person for such a central facility for all the different directions (plants, microorganisms form very different sources, marine organisms, biotransformation products) searching for novel biological active compounds.
3. The output in terms of IP (patents) is limited. Is this because of lack of novel interesting compounds, or lack of a routine/protocol to take care of first securing the IP rights before publishing. With other words the valorization of the results needs attention.
4. The years long waiting time for getting a license to collect certain plants is a major obstacle in the project and even worse is very much demotivating for (young) scientists. This means in the long term great economic losses for the Brazilian Society.
5. Qualified technicians for central facilities (e.g. large and expensive equipment, screening platforms) seem to be lacking, resulting in sub-optimal use of equipment and facilities.
6. It is not clear who has responsibilities for organizing platforms for e.g. the screening of biological activities. The group has expressed the wish to have more pharmacology, but who should take the initiative is not made clear. Considering the 2009 recommendations one may see that there has been made progress, but with all partners involved it will be difficult to make fast progress if not specific persons are appointed to take responsibility for deal with the various matters. The program coordinators do not have time to deal with everything. With other words a good management system is needed to improve and strengthen the collaboration.

Recommendations 2011

1. Build up a central platform for bioactivity testing, with a clear strategy for a well defined area of diseases (e.g. antibiotics, CNS, antiinflammatory, or anticancer) or other targets (biocides, dyes, cosmetics). This platform should also include preliminary toxicity tests (e.g. mutagenesis, cytotoxicity) for rapid identification of potential harmful compounds that have small changes for developing into a medicine.
2. Add in-vivo tests for advanced pharmacological testing of activities. This could include zebrafish and *C. elegans* as step in between in-vitro and animal tests. This would also be of interest to discover things like synergy in case of studying medicinal plants by using a systems biology approach.
3. A strategy for valorization must be developed, with many groups involved, the best would be a centralized office, which could be FAPESP or a private company that does the valorization on contract basis.
4. There is an urgent need to improve the system for getting access to material for bioprospecting. The (international) legislation for working with transgenic organisms

might serve as a model showing how to allow rapid progress in molecular biology and getting novel transgenic plants in the field.

5. Start to build up central libraries of microorganisms, extracts, gene sequences, enzymes, and compounds. The sinBIOTA database should have modules to search for this information in combination with the BIOTA database to be able to explore the biological space for interesting "chemical hotspots". This includes further development of the phylogenetic framework to facilitate exploration and assessments, in order to provide a solid basis of sustainable use of the biodiversity, and at the same time enhancing links between BIOTA and BIOprospecTA.
6. Concerning publications it would be good to show which groups participated in each paper to show to the outside world on the website how the program supports the collaboration between groups, to thus adding extra value.
7. One should consider the possibility make all output open access to increase the global visibility of the BIOTA project. It would require extra financial support to the groups when publishing in the open access mode, which is now offered by many journals.
8. Technical support personnel is needed for optimal use of equipment, both the granting organizations and universities should consider how to optimize the full time use of expensive equipment like NMR by enabling hiring qualified technicians. This is also important in connection with proper maintenance of equipment.
9. Organize at least once a year a (international) workshop on a novel technology or development that is of interest for bioprospecting, e.g. recombinatorial biochemistry, novel models for screening biological activity, metabolomics, metagenomics, toxicological testing. Such meetings will also lead to more interactions of the groups.
10. Dereplication is the keyword in the reductionist search for novel biological compounds. This requires libraries of physical data (e.g. MS, NMR or chromatography data) of known compounds and actives. This should be a central facility of the BIOprospecTA.
11. For central facilities (e.g. biological activity, libraries, NMR, MS) in the BIOprospecTA, the project should appoint responsible platform leaders that have the task to bring together the methods, collect/write SOPs for all users, and develop plans for further improvements and extensions of the platform. The platform leaders should advise the program coordinators about the plans and progess. With other words a clear management system of the BIOprospecTA program is required to further improve the performance and make optimal use of all the complementary expertise and equipment.
12. A central state-of-the-art sequencing center would be of interest for many BIOTA projects. The organization of such a facility in SP State where any project can pay for the costs of getting a gene or genomes sequenced with the latest method, would be of great use, and in fact an absolute requirement in the near future with the costs of sequencing decreasing rapidly. The sinBIOTA should be prepared for that in the near future the amount of information per species entry may dramatically increase with genomic, transcriptomic, proteomic and metabolomic data.

Final conclusion

The BIOprospecTA project is now in full swing, all participants with the same objective in mind. All the above mentioned observed weak points and recommendations are more meant to stimulate discussions to even further increase the quality and efficiency of the bioprospecting activities.

The major recommendation is that time has come to further increase interactions between the groups and to expand the program with groups in the area of pharmacology, toxicology, molecular biology and medicinal chemistry to go from hit finding to lead development. A clear management structure with responsible persons for various central functions should be helpful to optimize the interactions and to establish the different central facilities and keep these up-to-date.

My final remark is to congratulate the program with the excellent results, the international recognition achieved should be a great stimulant for all the researchers involved in the program to continue to strive for being best!

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