Latin-American Co-operation in Biotechnology Programme: Industrial penicillin amidase for 6 amino penicillanic acid production

Programa de Biotecnología para América Latina y el Caribe: Producción industrial de ácido 6 amino penicilánico utilizando penicilinamidasa

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ABSTRACT

This work evaluates technological and economic transference related to the Production of Penicillin Amidase for use in 6 Amine Penicillanic acid (6-APA) Production Project, wich is a part of the United Nations[1] Regional Biotechnology Programme for Latin America and the Caribbean. This paper analyses the evolution of international cooperation by looking at the project's development. The methodology used includes analysis of: the project's development; participant and budgetary goals; results; copyright; project conditions; and sale of biocatalyst and 6-APA. All technical objectives were achieved; international co-operation, as well as co-operation between Industry and University were successful. Technological transference to the pilot plant was effective; many students involved in the project were simultaneously taking M.Sc. and Ph.D's courses. Nevertheless, neither the technology necessary for the biocatalyst's manufacture nor the biocatalyst itself were used. Analysis of the project has provided some orientation concerning those internal and external problems which arose during the development and sale of biotechnology in our countries and has tried to propose some alternatives for taking advantage of international co-operation.

Key words: international co-operation, 6-APA, biocatalyst, immobilised enzyme.

RESUMEN

Se analiza la evolución de la cooperación internacional a través del desarrollo del proyecto "Producción industrial de ácido 6 amino penicilánico" desde el punto de vista de desarrollo tecnológico, transferencia de tecnología y su comercialización. Este proyecto se desarrolló en el marco del Programa Regional de Biotecnología para América Latina y el Caribe. La metodología incluye el análisis del desarrollo del mismo, los participantes, metas económicas, resultados, derechos de autor, las condiciones del proyecto, el desarrollo del biocatalizador, uso y comercialización. Todos los objetivos en la parte técnica, la cooperación internacional, la relación entre la Universidad y la Industria se llevaron a cabo de manera exitosa. Se hizo la transferencia de tecnología a nivel piloto, y muchos estudiantes de maestría y doctorado se formaron a través del proyecto. Sin embargo, ni la tecnología necesaria para producir el biocatalizador, ni el biocatalizador fue usado a nivel industrial. El análisis del proyecto intenta evaluar los problemas internos y externos que tienen que afrontar nuestros países para desarrollar y vender biotecnologías y propone algunas alternativas para alcanzar la comercialización de la tecnología y aprovechar mejor la cooperación internacional.

Palabras clave: cooperación internacional, 6-APA, biocatalizador, enzimas inmovilizadas.

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INTRODUCTION

Biotechnology is characterised by fast technical change, small innovative firms, sizeable expenditure on R&D, massive infusions of venture capital and rapid growth (Price, 1991). The developmental phase of any typical biotechnology's most promising products (therapeutic drugs and diagnostic assays for humans and animals) is subject to stringent regulatory procedures, many biotechnology products being aimed at those endusers or researchers who consume them. Established biotechnology corporations have begun to develop expertise in biotechnology so that they stay at the cutting edge (Freeman and Barley, 1990).

In 1987 international cooperation was focused on the creation of national biotechnology programmes to define policies for the development of modern biotechnology capacity in most Latin-American countries. With these programmes, governments orientated R&D Institutions and projects towards stimulating links with industry, channelling international co-operation and, in some cases, financing projects.

One of the main constraints confronting startup in Latin America is the lack of funding for R&D. Unfortunately, only a few startups were able to take advantage of financial support provided by government programmes. On the other hand, the scarcity of qualified personnel able to convert laboratory results into production on an industrial scale is the primary constraint on biotechnological development in Latin-America. Relationships between public research institutes and industry are poorly developed. Research is guided more by researchers[1] interests than by market demands, consequently the traditionally strong basic biological research on this continent does not result in useful agricultural or industrial application (Lawrence, 1994).

Governments from Latin-America and the Caribbean region agreed to provide assistance in carrying out the "Regional UNDP / UNESCO /UNIDO Biotechnology Programme for Latin-America and the Caribbean". Arising from this, the Regional Biotechnology Programme, the United Nations[1] Development Programme (UNDP), the United Nations[1] Industrial Development Organisation (UNIDO) and the United Nations[1] Educational, Scientific and Cultural Organisation (UNESCO) gave 5 million dollars for a biotechnology development programme in Latin-America. One part of the programme (for which UNESCO was responsible) focused on R&D up to laboratory level and on the improvement of education in basic science; the other part (being UNIDO's responsibility) was aimed at the detection and evaluation of suitable technology and consequent further development in pilot schemes for industrial application.

As Education is an integral element, then UNIDO decided to help the Industrial production of Penicillin amidase for use in 6 amino penicillanic acid (6-APA) production Project. The industrial use of enzymes was selected as being a priority area during the preparatory meeting in Havana (Cuba). There was a technical meeting to write the proposal for enzyme research and development. UNIDO, UNESCO and each country submitting its own programme formed the Regional Council for the Programme (RCP). This board analysed the results from all projects in 1987,1988,1989,1990 (Unido-Unesco).

When the project started in Latin-America companies were using both modern and traditional biotechnology (often engaging in traditional and modern biotechnology research at the same time). Biotechnological products are usually produced by traditional biotechnological means in Latin-America. Nevertheless, small businesses have been established by scientists associated with universities. It seems that the best possibility for the development of biotechnology (in Latin-American countries) is through established corporations involved in arrangements with research institutions (Quintero, 1990).

The project's first obvious strategy was defined as being technological development. This process pursued qualified personnel able to convert laboratory results into production at industrial level. On the other hand, it strengthened relationships between public research institutes and industry and tried to apply successful results to industry. This project was focused on carrying out policies for the development of modern biotechnology capacity in Latin-American countries, to stimulate links with industry and to channel international co-operation.

METHODOLOGY AND PROJECT DEVELOPMENT

Project goal

The project aims at providing manufacturing technology for penicillin amidase to be used in the production of hydrolysed penicillin G up to pilot plant. This process follows these steps: fermentation, enzyme purification, enzyme immobilisation and enzyme hydrolysis. Further steps involve hydrolysis product separation and purification and evaluation of possibilities of transferring the technology (to be developed in each participating country) to the industrial sector.

Criteria for defining the project's partners

The project has taken into account that 6-APA was not a new product, simply that the technology employed for production was new. A new product involves many chemical trials and bureaucratic processes and a sales[1] force must be established to launch effectively approved products into the market-place. When looking for partners for the project, it was very important to find out which countries were interested in the whole biocatalyst production process (and use) or in one of the components. México was proposed because Genin (from México) had been developing the process in the laboratory. Cuba had produced 6-APA chemically and this country wanted to change the technology used up to then. Colombia was interested in using the biocatalyst to produce ampicillin (for animals). These countries thus advanced the project; each country carried out a feasibility study and they considered that this process could have been profitable. They also had only one enzyme technology into the market.

This project's first activities went ahead in México, in 1987. Scientists from Cuba and Colombia were trained in the process in the laboratory; afterwards the group simultaneously worked on scale-up and biocatalyst characterisation. The 6-APA separation and purification processes were also developed. After the first year, working groups were organised in each country to replicate the results and develop other stages of the project. Technical meetings were held yearly to analyse results, control activities and decide on any changes needed.

During the whole time that industrial technicians were in training, students from different countries were taking M.Sc's. or Ph.D's courses. Also, during this time, some parts of the results were published. Most of the institutions managed to keep the same personnel on the project. Nevertheless, the project was late two years. The United Nations signed a contract with each institution on a yearly basis but, as each country's operational capacity was different, each organisation had to meet its respective requirements or the environmental and relative economic performance levels demanded.

Participants

The international co-operation agreement for Colombia, Cuba and México involved five institutions from these countries.

Colombia: Vecol is an established semistate Corporation. This is a leading company for veterinary and agrochemical products (biological and/or chemical production). Vecol was interested in using the biocatalyst. Vecol has its own R&D department, involving agreements with university and research institutes. The Universidad Nacional of Colombia's Biotechnology Institute (public university institute) carries out basic and applied research.

Cuba: Imefa (Industrial Union for Medical and Pharmaceutical products) is a company producing ampicillin chemically. This industry was interested in exchanging technology (it has now changed chemical production for enzyme production). The Cuban Biotechnology Centre carries out basic and applied research.

México: Genin S.A. is a new biotechnology business; it is a private company, but lacks production facilities. This biotechnology firm was funded to pursue applied research in enzyme technology. It was founded by Mexican scientists associated with UNAM (National Autonomous University of México). This company developed the biocatalyst technology at laboratory and promoted the project.

Process characteristics

Penicillin and its semisynthetic derivatives are the most used antibiotics in the world. Natural penicillin is produced by fermentation. A large quantity of different penicillin can be produced by introducing different chemical groups into amino penicillanic acid (6-APA).

Many kinds of penicillin (for use against different micro-organisms) have been created in this way.

6-APA world production was 3,000 to 4,000 tons in 1984, more than fifty percent of this resulting from enzyme production. In 1990, the demand for 6-APA was 5,250 tons and it was hoped that by the year 2000 the demand would have been 7,000 tons (Buitrago, 1990). The principal semisynthetic antibiotics produced by using 6-APA are ampicillin and amoxicillin (2,350 tons and 1,150 tons respectively every year).

At present, there are two ways of producing 6-APA (i.e. by chemical and enzyme processes). The chemical process needs special temperature and solvent conditions; it is also more difficult and more expensive (Quintero, 1986). The enzyme process (Figure 1) requires less drastic conditions and a watery medium. Nevertheless, the critical aspect in this process is the cost of the penicillin acylase enzyme (biocatalyst).

Project co-ordination

United Nations' Organisation for Industrial Development (Unido) coordinated the Biotechnology Programme for Central and South America through its director and also signed yearly contracts with each institution. The project's detailed activity programming was defined monthly. This project had a means of gauging results as the project director organised a yearly meeting to analyse the results, control the activity programme and define the terms of reference for new contracts.

Each country selected personnel for the project. In the first year, all scientists were working in Genin, México, and conditions for the research were simultaneously established. The scientists applied different skills to ideas as a result of their own backgrounds. After the second year, the work was carried out in each country and all pilot plant experiments were done in México. The scientists from Colombia and Cuba were in México during the project's development stage.

Budget

The project was financed by the United Nations[1] Organisation for Industrial Development (UNIDO), under an International Cooperation Agreement ¡n Colombia, México and Cuba (presently represented by five institutions from these countries).

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Venture Capital: Imefa and Genin -US$120,000.

Facilities were provided by government institutions. The Universidad Nacional de Colombia's Biotechnology Institute and the Cuban Biotechnology Centre paid for the personnel. One laboratory and other facilities were adapted for the project's needs.

Results

During the project, the following objectives were achieved: a stable E. coli strain was obtained, the industrial culture medium was optimised and the fermentation process was developed (temperature, pH., aeration, process time). The parameters were scaled up to 500 L. The yield (Y s/p) was 4.04 g/l for cells with 110 U/g; in production, enzyme recuperation and purification was 450 U/L. Total efficiency was 25% after cell disruption, precipitation and concentration. The enzyme was purified by fractioned extraction, ultrafiltration and ionic interchange. The immobilisation process was developed. Support capacity and reaction conditions were defined (pH, temperature, ionic force and time). Following these steps, 45% to 50% of the enzyme was recovered; biocatalyst activity was between 140 to 170 U/g. Hydrolysis conditions were defined. It is possible to use the same biocatalyst for more than 500 batches. At these conditions, there was a 95% conversion rate. 6-APA purification was developed. The above information has been taken from the project's technical reports.

The feasibility study has shown that industrial 6-APA production depends on PGK's International price. 6-APA production is not profitable ¡n any of the participating countries, because it is necessary to purchase imported penicillin (Buitrago, 1990). The quality of biocatalyst obtained in this project is similar to that of other products on the market.

Copyright

The technology has not been patented. Some parts of the results have been published, nevertheless, the technological package is not going to be made available

to the public. The participating countries have decided to protect it as an industrial secret. Unido has agreed with such decision. The rights will be distributed equally amongst the participating countries and their respective institutions (Workshop, 1993).

Biocatalyst and 6-APA commercialisation

This project, aiming at putting efforts together from three countries, would involve three companies and two research institutes. The project was defined as being technological development to use the experience gained in the process at laboratory in Genin, México (NBFs). It was known when the project started in 1987 that the economic model in force in Colombia was Importation Substitution, employing economic protectionism. The principal objective aimed at developing the domestic market in order to strengthen Colombian industry. In Cuba, the economic model was completely state dominated. At the same time, México had embraced the open market.

By 1992 the economic situation had changed: México had entered Nafta and Colombia had adopted the Neoliberal model; these countries are members of different economic blocks. Feasibility studies in Colombia, México and Cuba were made, based on these conditions. The industrial production of penicillin amidase, for use in 6 amino penicillinic acid (6-APA) production, was not feasible. Chemical and pharmaceutical products thus became subject to open market laws.

As a variety of business issues are involved in the commercialisation of that technology necessary to manufacture the biocatalyst and 6-APA, Unido funded a project group which presently includes México, Colombia and Cuba. At the Vienna workshop (April 25th-April30th, 1993) it was decided to establish a formal consortium amongst these three countries (as represented by participating institutions). The formal creation of a business consortium was agreed upon, in principal, with each country receiving one third share or interest in the activities necessary to initiate the commercialisation of biocatalyst and 6-APA. Progress has been made and discussed concerning issues related to ownership of the technology and coordination of commercialisation efforts.

The purpose behind the desire to commercialise this process would be to accelerate Latin-America's

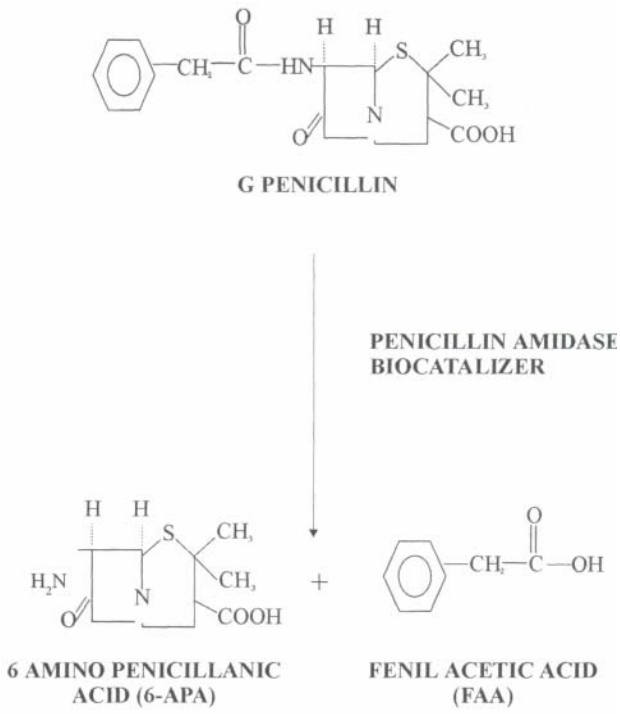

Figure 1. Enzimatic reaction for the 6-APA production.

selfsufficiency, not only in enzyme production, but in those processes necessary for the creation and sustainability of a biocatalyst and 6-APA market for countries which presently import most or all of their synthetic penicillin requirements. The business plan should be developed to broadcast the basic biocatalyst and 6-APA process's commercial objectives to interested parties. The plan would need getting national and regional involvement in determining the financial viability of commercialising the technology.

DISCUSSION

This programme (operating within the Biotechnology processing industry, characterised by relatively rapid technological change) has produced modifications and innovations. Changes in scientific knowledge and continuous innovation might outdate present methods and products. The rate of change in the market is relatively high. A high extent of integration is required, primarily because of the necessity for developing a new process. But, requirements for effective integration between sales and research are important. The ideal approach in biotechnology is an integrated one, where marketresearchers and product research groups work together to asses market needs and develop the plan

for producing and marketing the products (Powell, 1995).

The project was designed to define an identifiable nichemarket strategy. This strategy had to be able to dominate a small market and avoid competition in the larger ones. The segment has been diagnosed and has been wellestablished in the market for the past 10 years, with product sales providing the majority of revenue for the project's firms. The business consortium was created in order to resolve two problems; it agreed upon the principal that each country should receive one third share of income deriving from the project and was interested in those activities necessary to initiate biocatalyst and 6-APA commercialisation. A business plan had to be developed to spread the basic commercial objects for the biocatalyst and 6-APA processes to interested parties. Such a decision was adopted following feasibility studies in each country. External or internal forces affecting the project's ability to serve this technology market were considered. An attempt will be made to interpret this project, using Price's analysis framework (1991).

Usually, a substantial amount of biotechnology safety regulation accompanies new products or technology before they come onto the market, but, with this product, being the raw material for ampicillin production, productlaunch was accomplished in the minimum necessary time. It had just been characterised and the process's products were known. But, in the Colombian case, the principle concerning commercialisation was the problem, as this involved a governmental (i.e. political) decision. When the project started, those participating countries were employing the protectionist economic model; when the project ended, México and Colombia had adopted a neoliberal model and Cuba was open to foreign investment. Thus the strategy orientated towards dominating a small market and avoiding competition ¡n the larger market was affected. It is clear that government macroeconomic politics have left many projects with no economic profitability.

It was very difficult to detect the competition because the project did not have a special team to study the product on the market. This project began with product output research and development and continued on the same lines with development, marketing and sales. It might have been possible to detect the influence of other technology development on the larger market by using a team dedicated to this area. The second aspect dealt with internal forces. This was successful ¡n terms of the programme itself, as it led to control being implemented over project development. The coordinator was able to develop subsystems in each country and manage systems in each participating country.

The team within the project was to facilitate various organisations[1] activity coordination by providing formal tools for discussion and mutual problemsolving. Effective coordination was developed amongst research groups (orientated equally towards longterm and short-term problems) for achieving the scientific and technical goals. Similarly, group interpersonal orientation was coordinated and linked to subsystems[1] basic function structure ¡n each country and to that which was functional in the countries, resulting ¡n a high degree of integration.

Another internal and positive force (in this case) was the project's ability to raise enough capital to fund research and development. But, if it had taken advantage of international cooperation, it would have been ¡n a better position to understand economic development and market strategy. It was important that the project should obtain not only technical, academic and organisational success, but also economic benefits.

By contrast, technologists' and behavioural scientists' work has focused on technology through economic analysis. In this project, product and goal selection were correctly identified. Nevertheless, the project's development took eleven years, including laboratory and scaleup levels. During this time, innovation was affected by being forced into competition with new products and technology. Thus, the product (developed in this project) was standardised, the technology was efficient and the area for competition lay primarily in raw material (penicillin) price. But, currently, the problem lies in the highly integrated systems on the market, because the companies producing penicillin also produce the catalyst. The reduction in production costs for these products has been the result of a gradual integration and improvement process. A key lesson was learnt from the experience relating to this project; it was necessary to understand others[1] needs and be able to appreciate others' experience to reach agreement concerning new product development in the short term.

Effective project management must integrate horizontal functions and carry out upfront R&D, marketing, manufacturing and management, thus facilitating successful product development, as stated by Wheelwright (1988). The relationship between individual organisations and their immediate environments (as a result of research giving more attention to organisations) was constrained by current events or by relationships with organisations which they had no ties with.

Latin-American countries should think about a new strategy for developing technology; this strategy could consider establishing links with small companies in developed countries and taking advantage of financial support provided by government programmes and international cooperation. It could be a good strategy to seek out companies having products either complementing or extending one's own lines. The final objective is to reduce new product development costs and guarantee a market positioning.

CONCLUSIONS

In 1986 the Industrial Production of 6 amino penicillanic acid (6-APA) Project was defined as being technological development. From the start, a Mexican company (Gening) had experience in a process which was in a stage of gestation all around the world and (at the time) there were opportunities for transferring this technology to the industrial sector.

The project could have developed a strategy for market entry in Latin America, where the project started. This could have been explored with a potential investor, jointventure partner and/or government. The development of a business plan and promotion of the technology to target audiences would have generated various business options. Unfortunately, the project was straightjacketed by the definition of a nichemarket strategy aimed at dominating small markets in Colombia, Cuba and México (not foreseeing macroeconomic change in the short term).

This process needs human resources, technical and economical organisation; these conditions were difficult in each country, particularly in the 1980s. However, in this setting, the underlying support and timing were important because the countries were able to work together and learn and develop skills to put together a technological package. This skill is important when technology is an important variable.

The project was to finish in 1990, but it really ended in 1992. By the time the product reached the market, it was no longer at the cutting edge (even though the technology used was similar to others on the world market), so it becomes clear that it must also be developed on time. During the time taken for development (1987-1992), innovation was affected by competition, new products and technology. If advantage of international co-operation had been taken, then there would have been understanding of economic development and market strategies.

Latin-American countries should consider new strategies for developing technology, i.e. they could consider forging links with companies from developed countries and develop at least a part of the necessary technology. Countries would thus not be far away from being at the technological cutting edge, and could take advantage of biodiversity and financial support provided by governmental programmes and develop technology through international cooperation.

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