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SUMMARY - The Yugoslav country has benefited from a long-standing tradition in the field of biotechnology. New biotechnologies, essentially based on recombinant DNA, are of a recent date. Basic and applied research is far more developed than transfer of knowledge as far as new technologies are concerned. Scientific research is unevenly developed. Research focuses more on plant than on animal production, thus more applied results are available concerning the former. In Yugoslavia, research concentrates on two major projects: GIBIT (Genetic Engineering and Biotechnology) and Biotechnology of the Future. In spite of some autonomous projects existing in the republics and large enterprises, it can be stated that both of the above-mentioned macro-projects at the Yugoslav scale, are the framework impulsing research in biotechnology. The GIBIT Project is financially supported by all prominent self-managed Communities in the field of Science at the level of every Yugoslav republic and province. The content of the Project comprises mainly basic research in the field of biotechnological systems based on genetic engineering, specialized basic research for the requirements of GIBIT, the construction of new strains of microorganisms, by improvement of biotechnological methods, the application of GIBIT products and methods in prophylaxis, diagnostics and therapy in human and veterinary medicine, aiming GIBIT at agriculture and forestry, at biological transformation in industrial applications, fuel production and environment protection, and eventually the development of an information sector for GIBIT. The biotechnology of the future is a project of recent creation in Yugoslavia, and 40% is financed by the Fund for Technological Development of Yugoslavia. An international cooperation is required in the area of biotechnological research. Every research institute is participating in intensive international cooperation, and must increase this exchange. There is a lack of competent staff at every level of the biotechnological field, and research for an increased production is needed. It is thus necessary to take the appropriate steps to ensure training in every area of the country, and abroad in the most advanced centers. Results, still individual, would encourage a fast introduction of new methods in order to increase food production, and especially production of health food. It must be therefore noted that biotechnological methods are complementary to traditional ones, and by no means a substitute to them. New biotechnological methods are then adequate to improve some traditional technologies and to increase their effectiveness.

Key words: Biotechnology in Yugoslavia - Molecular biology - Fermentation - Biomass - Biosynthesis - Gene manipulation - Tissue culture - Gene vector - Genetic transformation - Technology tranfer - Training.

RESUME - "La stratégie développée par la Yougoslavie en matière de biotechnologie". La Yougoslavie bénéficie d'une longue tradition en matière de biotechnologie. Les biotechnologies nouvelles, basées essentiellement sur l'ADN recombinant, sont récentes. Les recherches fondamentales et appliquées sont bien plus nombreuses que les transferts des connaissances dans les technologies nouvelles. Le travail de recherche scientifique n'est pas développé de manière égale. Les recherches sont focalisées plus sur les plantes que sur les animaux, d'où davantage de résultats appliqués dans la production de plantes. La recherche au niveau de la Yougoslavie est concentrée dans deux principaux projets : GIBIT (ingénierie génétique et biotechnologie) et Biotechnologie de l'avenir. En dépit de l'existence, au niveau des républiques et des grandes entreprises, de projets autonomes, l'on peut affirmer que les deux macro-projets cités au niveau de la Yougoslavie constituent le cadre de soutien de la recherche dans le domaine de la biotechnologie. Le projet GIBIT est financé par toutes les Communautés d'intérêt autogestionnaires en matière de sciences au niveau de toutes les républiques et provinces yougoslaves. Ce projet consiste principalement en recherches fondamentales dans le domaine des systèmes biotechniques basés sur l'ingénierie génétique, en recherches fondamentales spécialisées pour les besoins du projet GIBIT, en construction de nouvelles races productives de micro-organismes, en promotion de procédés biotechnologiques et obtention de produits nouveaux, en application des produits et des méthodes GIBIT dans la prophylaxie, le diagnostic et la thérapeutique de la médecine humaine et vétérinaire, en orientation du projet GIBIT vers l'agriculture et la sylviculture, vers la biotransformation appliquée dans l'industrie, en production de combustibles et protection de l'environnement, et en mise en place d'un système d'information pour les besoins du projet GIBIT. La Biotechnologie de l'avenir est un projet récent au niveau de la Yougoslavie, financé pour environ 40% par le Fonds d'encouragement du développement technologique de la Yougoslavie. Ces deux principaux projets absorbent, on peut le dire, le gros des potentiels scientifiques et de recherche dans le domaine de la biotechnologie en Yougoslavie.

La recherche biotechnologique impose une coopération internationale. Tous les instituts de recherche yougoslaves entretiennent une coopération internationale poussée qu'il y a lieu d'intensifier. Le personnel qualifié pour tous les échelons de la biotechnologie, de la

recherche à la production, est déficitaire. Aussi est-il nécessaire de prendre les dispositions appropriées en vue d'assurer toute sorte de formation dans le pays et dans les centres les plus développés à l'étranger. Les résultats obtenus, encore individuels, viennent encourager une introduction accélérée des méthodes nouvelles dans l'augmentation de la production d'aliments, et notamment d'aliments sains. Il est, toutefois, à noter que les méthodes biotechnologiques représentent un complément des technologies traditionnelles et non leur substitut. Les nouvelles méthodes biotechnologiques peuvent effectivement ennoblir certaines technologies traditionnelles et en accroître l'efficacité.

Mots-clés : Biotechnologie en Yougoslavie - Biologie moléculaire - Fermentation - Biomasse - Biosynthèses - Manipulations génétiques - Culture de tissus - Vecteur de gènes - Transformation génétique - Transfert de technologie - Formation.

Introduction

A more efficient food production is indispensable to satisfy the needs of a growing global population. Health protection in an ever more polluted environment is also an imperative of the present time. One of the foremost methods to preserve health is the production and use of health food.

Agriculture can be considered as the most important of human industrial activities since its products affect immediately and directly the well-being of mankind.

Agriculture is both the oldest and largest of the world's industries and it will remain so for the foreseeable future.

Dramatic gains in agriculture of Yugoslavia have been made in the last three decades largely as a result of the intensified use of land, fertilizers and pesticides, and by planting improved crops developed through very extensive and efficient breeding programs.

It is to be expected that, among many other measures, biotechnology will provide an impetus to increased food production and maintain the trend of advancement in various fields, particularly in plant breeding. Yugoslavia has no reservations in expanding agricultural land. New potentials for food production are enhanced efficiency of land utilization and new methods in food production.

Biotechnology offers a number of possibilities complementary to traditional agriculture. This has been the subject for many papers. Similarly to other Mediterranean countries, biotechnology holds special significance for Yugoslavia as its climatic and soil conditions enable an almost permanent high production of raw materials for biotechnology. Besides using those comparative advantages of the Mediterranean, biotechnology could be important to bridge the gaps in the range of agricultural products of interest for the country's economy and for export.

Traditional biotechnology has a long-standing tradition in Yugoslavia. In order to provide an insight into traditional biotechnology, I shall quote only a few of its products:

- *Fermentation products* (beer, wine, ethanol, natural strong alcoholic beverages): Annual beer production (in 30 breweries) attains 12.5 million hectolitres. There are realistic prospects of increasing production within the facilities available by 20 to 30%. Viticulture is well-developed in Yugoslavia. Hence, wine production is an important branch of economy. Annual production totals about 3.66 million hectolitres of wine. It fluctuates depending on policies in agriculture and sociological changes in rural areas. Ethanol production is very modest (approximately 60,000 t/year). Its production is primarily based on molasses which are in short supply. Therefore, production of absolute and industrial alcohol and refining capacities are insufficient. The use of starchy raw materials (potato and maize) is associated exclusively with vodka production. Natural strong drinks (natural brandy) include primarily plum and grape products. Annual production is about 145,000 hectolitres. In spite of a modernization and industrial production, there is substantial household production, which is hardly rational.

- *Production of microbial biomass:* Microbial production is very important as a food source due to food deficiency in the protein component. In Yugoslavia this production is not developed.

The quantity of baking yeast produced is about 50,000 t. The production is based on licensing and the basic material is molasses.

Single cell proteins (SCP) could possibly give a sizable contribution to solving the protein deficit in food and feed. In Yugoslavia SCP is lacking, above all

in primary production. Fodder yeast is produced along with ethanol (Vogel-Bush technology) exclusively on molasses as the raw material.

- *Aminoacids:* The classical method of obtaining aminoacids cannot meet the growing demand due to its limitations and constraints. Microbial synthesis has a number of advantages. Although technology has been available since 1978 and so have been the 26,000-ton facilities for the production of L-lysine by microbiological synthesis, Yugoslavia does not produce any aminoacid in larger quantities on a commercial basis to satisfy the growing demand of many branches of economy.

- *Organic acids:* Among the organic acids Yugoslavia produces citric and acetic acids, and to a more limited extent gluconic and technical lactic acid. The quantities of citric acid produced using the biotechnological method reach about 4,000 t/year. This quantity lags behind the requirements of Yugoslavia. Production of technical lactic acid is far below the needs of the country (about 1,000 t annually). Vinegar production is slightly higher (about 40 million litres per year).

- *Fermented food products:* These products (yoghurt, cheese - 10,000 t/year, etc.) represent traditional food. In most cases production is based on craftsmanship rather than industrial technology. Hence, in the conditions of industrial production (except for some dairy products) they do not provide superior quality.

- *Natural antibiotics:* Although it produces only seven of the 90 natural antibiotics manufactured in the world, Yugoslavia ranks high. In world production Yugoslavia accounts for 25, 12 and 1.5% of global bacitracin, oxytetracycline and tetracycline, and penicillin G production, respectively. It is our opinion that there are additional capacities for further increases in antibiotic production in Yugoslavia.

- *Production of vaccines and blood derivatives:* Vaccine production for human and veterinary purposes is highly developed. The major manufacturers include the Immunological Institute, Zagreb; the Torlak Institute of Immunobiology and Virology, Belgrade; Pliva, Zagreb; INEP, Belgrade, etc.

- *Enzymes:* Enzymes are already used in food industry (beer, wine, fruit juice and high fructose corn syrup production) and leather industry. Enzyme production in Yugoslavia is at its initial stage. Negligible quantities of pectinases ("TOK", Ilirska Bistrica) are manufactured. The production of the commercially most important hydrolytic enzymes - amylase and proteinase - does not exceed 50 t/ha/year. The demand is as high as twentyfold, so high amounts of enzymes are imported.

- *Inoculum production and its use in legumes:* The inocula produced are most commonly used for soybean inoculation. The areas under soybean are entirely planted with inoculated seed.

Inocula for other legumes (peas, beans, alfalfa, red and white clover, yellow lotus, etc.) are produced and used in much lower quantities.

These are some of the traditional products in which biotechnological methods were also employed. Although the above production data and the demand for those products are incomplete, they indicate the basic developmental policies .

These products are promising. The industry will improve and increase them.

Policies in Biotechnology and its Association with Classical Science

In many fields the efficiency of traditional biotechnology has proved to be insufficient.

Progress is foreseen for some products quoted in the introduction. It mostly refers to research and can be defined as follows:

- The possibility of obtaining biological raw materials for *ethanol* production. Primary raw materials (maize, sugar beet, hybrid sorghum) are studied separately from secondary raw materials (straw, stover, waste material from agriculture and forestry).

- Basic operational and technological research of acid- catalyzed hydrolysis of lignocellulose materials for obtaining basic substrates (sugars).

- Development of associated technological processes of simultaneous ethanol and protein animal feed production from lignocellulose materials obtained by acid-catalyzed hydrolysis.

- Genetic manipulations and selection of new strains of thermophilic yeast for fermentation of C5 and C6 sugars into ethanol with a high ethanol tolerance in fermented husks.

- Selection of highly active and stable yeast strains in the production of *microbial biomass.*

- Due to a deficit in molasses, studies of a possible use of alternative raw materials for yeast production.

- The production of *organic acids* does not yet include on a large scale the modern technological procedures (submersal

production), so the classical Frings equipment must be replaced entailing sizable investments.

- The production of *natural antibiotics* most easily accepts the methods of genetic and biochemical engineering, since pharmaceutical industry has developed research and development centres.

- The production of *inocula for legumes* comprises more active bacterial strains, plant breeding, identification of more efficient inoculation methods to more successfully suppress endogenous strains and expand the application of inoculants to the majority of legumes grown in Yugoslavia.

The Coordination Committee of the Council of Yugoslav Academies of Science for Genetic Engineering and Biotechnology has defined the following guidelines in genetic engineering and biotechnologies:

- Modern biotechnology is inconceivable without pure culture of *microbiological collections.* Nowadays even traditional technologies (beer, cheese, wine, etc.) use only pure cultures. Due to an uncertainty of steady imports of the best strains, breeding activities based on genetic engineering have to be established.

- *Genetics of microorganisms* entails studies of the organization, structure and functioning of the genome of microorganisms, investigating and inducing mutations on a specified site in the DNA molecule, removing bottlenecks in biosynthetic pathways with different metabolites - by gene cloning, extending the capacity of microorganisms to grow on new media.

- *Application of genetic engineering in the field of vaccines, plasmaproteins and diagnostics.*

- *Physiology of microorganisms,* primarily those used in industry.

- *Genetics, molecular biology and plant physiology.* In addition to highly developed breeding, intensive studies are carried out on the use of genetic engineering. A major contribution of new technologies is given in extending genetic variability, which is the principal prerequisite for plant breeding, protoplast fusion, vegetative micropropagation in vitro, problems of nitrogen fixation, improvement of plant resistance to stress conditions and improvement of plant resistance to pests and studies of physiological processes in the plant.

- *Enzymology and enzymatic engineering* (an economical source of enzymes, knowledge of separation methods and protein analysis, knowledge of protein chemistry, physical, chemical and catalytic characteristics, and the structure and mechanism of enzyme action).

- *Studies of alternative raw materials* (molasses, starch and sugar raw materials, plant mass and waste lignocellulose material, waste organic materials in food and fermentation industry).

- *Development of biotechnological equipment* (bioreactors and instruments, automatization and control of processes).

- *Comparative studies of modes of monitoring processes.*

- *Mathematical* modelling (kinetics of microorganism growth and biosynthesis of microbial metabolites, biochemical reactors, experiment planning, development of mathematical models in detecting genetic structures, development of computer programs for process emulation, use of computers in process monitoring).

- *Bioinformatics* and international cooperation.

- *Legislation.* Biotechnological products are subject to legal control involving specified standards, identity, activities, quality and purity. Defining these standards requires an elaboration of appropriate analytical methods and determination of a list of specifications in order to identify acceptable limits for each characteristic tested.

Many international organizations have developed specifications, but legislation has to be adjusted accordingly.

Protection in biotechnology is of great importance for each country and should be approached as an international problem.

Coordination and Support

All these guidelines of the Coordination Committee are partly or entirely implemented through two large-scale projects which basically support research in biotechnology (GIBIT and Biotechnology of the Future).

The Yugoslav *GIBIT Project* (Genetic Engineering and Biotechnology) has been established. This project is financed by research associations of all republics. It has its project council. The data and progress reports are submitted to the Council of Research Associations of Yugoslavia.

The content of this project comprises the following fields:

- Basic research of biological systems based on genetic engineering.
- Specialized basic research for the requirements of GIBIT.
- Construction of new productive strains of microorganisms.
- Improvement of biotechnological procedures and obtaining new products.
- Application of GIBIT products and methods in prophylaxis, diagnostics and therapy in human and veterinary medicine.
- Aiming GIBIT at agriculture and forestry.
- Biological transformation in industrial applications, fuel production and environmental protection.
- Development of an information sector for GIBIT.

All areas include research that directly or indirectly can refer to agriculture, but for a better understanding of the level or research, that part of the program will be presented which involves more specifically agriculture.

- Evolution of the nuclear genome in the growth and development processes. The objective is a cheaper and more rapid detection of mutagenesis and action of synthetic growth regulators and pesticides.
- Application of plant tissue culture in genetic engineering. The possibility of cell transformation in potato tuber has been studied using several strains of the bacterium *Agrobacterium tumefaciens*. Conditions have been defined for cloning of the pumpkin shoot and somatic embryos in vitro. The objective was to assess the difference between normal and transgenic roots and tissue using the analysis of nuclear DNA and to identify conditions that enable shoot induction in transformed tissue, which is a prerequisite for obtaining plants with new properties.
- Microcloning of vine and vine substrates. It is aimed at identifying genotypes in various conditions for callus formation in meristem zones or embryonic tissue to determine whether regeneration of adventitious shoots and/or somatic embryos is possible.
- Application of tissue culture methods in sunflower breeding. The objective is to overcome the problem of incompatibility in interspecies hybridization and to accelerate the breeding process.
- Tissue culture in peach breeding. In addition to increasing efficiency of shoot regeneration methods from immature embryos and cotyledons in vitro, the objective was to obtain varieties resistant to diseases, primarily to peach pox virus.
- Crown gall induction, hormone metabolism, morphogenesis. The ultimate aim is to grow by micropropagation a great number of transgenic buckwheat plants and to study the characteristics of transgenic plants.
- Studies of somatic maize embryogenesis.
- Haploid and diploid cereal cultures. The objective is to identify and improve growing practices for dihaploids by anther growing in order to obtain homozygous lines.
- Identification, isolation and cloning of maize genes that control stress resistance. It has been established that optimal sources of carbon for two strains of Kabatiella zeae (pathogen of a maize leaf disease) are sucrose and d-glucose. Genetic variability of maize in carbohydrate content and composition enables identification of genotypes with a different plant response to the pathogen - a differing level of gene expression. The objective is to isolate the genetic construction that contains the control-transposon elements.
- Assessment of optimum vector systems for gene incorporation in tissue, cell or plant organism culture. It was found, in F5 generation of maize plants transformed by incorporating the bacterial gene which controls resistance to kanamycin that, by injecting the bacterial DNA, transformation was made of both the maize and bacterial genomes that are normally situated in the cytoplasm of maize cells. Further studies questioned whether the plant contains the original DNA (*E. coli*) or whether it was the bacterium that lives in the plant. The strain was found to be new. The patenting procedure for the bacterium and plant genome transformation is in progress.
- Biological fixation of atmosphere nitrogen. The objective is selection of strains of free nitrogen fixers and wheat varieties.

- Competitiveness of *Rhizobium* spp. strains. The objective is to select the best among the new mutants.

"Biotechnology of the Future" is a project at the federal level, of a recent date. It is financed from various sources with 40% from federal funds intended for support to technological development. The title is quite bombastic, probably resulting from a desire to emphasize work mostly based on genetic engineering.

The content of this project is also composed of basic and applied research:

- Construction of new strains of beneficial microorganisms
- Study of the mechanism of spontaneous and induced genetic changes
- Biosynthesis of biologically active compounds
- Molecular genetics of streptomycetes
- Hydrolytic enzymes and their inhibitors
- Development of modern bioconversion procedures
- Mechanism of glycoprotein expression in yeast
- Bacteria of lactic acid fermentation with specific properties
- The functionality of modified acid-lactic bacteria
- Molecular genetics and genetic engineering of prokaryotes
- Biologically active proteins and their possible production using biotechnology methods.

Almost the entire research staff of Yugoslavia in this field is concentrated on these two major projects. Financial support to the projects implies certain coordination. However, there is no federal body that exclusively deals with biotechnology. All research projects are coordinated through the association of research associations of the republics and through the Federal Secretariat of Development. In addition to these major projects of Yugoslav (national) character, there are autonomous research and development projects in the republics and large enterprises.

Resources

The ambitious program in biotechnology lacks sufficient competent staff, although in some fields highly specialized scientists are available (molecular biology and recently genetic engineering). Organized training from the manufacturer to the research scientist is necessary. The specificity of biotechnological products requires specialized staff for product control. Financing is limited and organization without firm coordination.

There is a growing awareness of the need to introduce biotechnology into food production with the objective of increasing food production, particularly that of health food, which is in high demand. There is a trend of introducing biotechnology into many other production areas as well, especially into environmental protection.

This approach has been reflected in the strategy of the technological development of Yugoslavia, which was adopted by the Federal Parliament three years ago. One of the technologies that will receive priority in support and development will be biotechnology.

Due to the difficulties experienced by Yugoslav economy, few firms and individuals invest in new biotechnologies. This particularly refers to investments in facilities for biotechnology and investments in research. There is also the problem of transfer of the results: from science to technology and production. There are several underlying reasons, which are identical to the general relationship between science and production. Yugoslav science is characterized by inadequate distribution of research staff (about 80% are at universities and independent institutes and only 20% in enterprises). Adequate transfer of knowledge from research institutions to production requires well-organized and developed research and development in commercial enterprises. Another reason is the risk involved in new production (technology), which for the time being is the risk assumed only by the enterprise. Risk funds have not been created concerning introduction of new technologies based on one's own knowledge. Such possibilities will soon be offered by the banking system.

International cooperation

Production in "classical" biotechnologies is largely dependent on foreign licenses. This mode of "cooperation" has not been synchronized. Free enterprises have independently carried out purchases abroad with insufficient competence, without their own development making the right choice and the development of purchased technology impossible. Therefore, such "cooperation" has frequently been unsatisfactory. Export-oriented enterprises, which as a rule have a highly developed development sector, have purchased competently the latest technologies, adapted and developed them thus increasing the level of their development and research.

Scientific and technical cooperation has a different character. Many research institutions have a very developed scientific cooperation ranging from exchange of information, scientists and data to joint projects. However, such cooperation also demands more favourable conditions, primarily training programs for scientists in the most advanced world centres. An international environment that would favour a more open cooperation, more liberal exchange of technologies with less restrictions is necessary. This particularly refers to biotechnologies in environmental protection. Certainly, open cooperation and transfer of technology need a unified (to the extent possible) relevant legislation. One could think of creating ways to use the International Centre of Biotechnology and Genetic Engineering in Trieste, as well as of developing a permanent school for certain fields of biotechnology. We would readily support such training.

Conclusion

The development of biotechnology is no longer spontaneous and accidental. Various institutional policies, from the Strategy of Technological Development of Yugoslavia, where biotechnology receives top priority, the Act on Establishing and Using Resources for Support to Technological Development, establishment of major projects, incorporating almost the entire scientific potential in this field (GIBIT and Biotechnology of the Future), creation of molecular biology centres and genetic engineering centres and better managed training are promising for advancements in biotechnology.

Research projects are at a relatively high level, but transfer of science into new products has many shortcomings.

Although there is a growing awareness of its importance, impeded development of biotechnology may result from an insufficient number of trained specialists, scarce financial support and scant presence of research scientists in enterprises.

The results attained, still sporadic, encourage a more rapid introduction of new methods into production. However, biotechnological methods are a complement to the traditional ones and by no means their substitute. New biotechnological methods can improve and increase the efficiency of some traditional technologies.

Considering the rapid pace of development of science and production in this field, international cooperation is of utmost importance. It should receive more attention both in bilateral and multilateral relations. This refers to all modes of scientific and technical cooperation from exchange of information, exchange of specialists and training programmes to joint research projects.