FORETECH – TECHNOLOGY AND INNOVATION FORESIGHT FOR BULGARIA AND ROMANIA

BACKGROUND PAPER ON BIOTECHNOLOGY

DEVELOPED BY
ARC FUND
IN COLLABORATION WITH
BIOTECHNOLOGY EXPERTS
FROM KEY STAKEHOLDER INSTITUTIONS IN BULGARIA

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BIOTECHNOLOGY FORESIGHT IN BULGARIA (BACKGROUND PAPER)

I. Introduction

This document presents a critical survey on past developments and the present state of biotechnology in Bulgaria, with the aim of outlining the areas most relevant for foresight activities.

There have been three distinctive periods in Bulgaria's biotechnological development. The first, started in the early 1980-ies when Bulgaria set biotechnology as a policy priority. The country laid the foundations of modern biotechnology during that stage and invested heavily in education, R&D and application. The second stage, which began with the collapse of the planned economy in 1989, saw a sharp decline in biotechnological financing, disruption of existing institutional links and loss of assets, following the general economic problems of transition. It was not until 1997 that biotechnology began its gradual recovery, led by privatization and an inflow of foreign direct investment into the sector, swift macroeconomic stabilization and the emergence of new small private companies. This rebirth, building on traditional strengths – production facilities and educated labor force - will now be shaped by the country's EU accession and reintegration into the world economy. Thus the timing of the proposed foresight exercise fits suitably into the natural development of the biotechnology sector in Bulgaria.

Although there is good ground to expect a rising development in biotechnology in Bulgaria there are a number of challenges that companies face in this area. On the top place comes the lack of appropriate financing instruments, followed by an outdated legislative base and unstable property rights regimes. These issues will find part of their solution in the countries' EU accession, which will bring in stability and predictability, as well as more financial resources. On the other hand EU accession will pose new challenges, not least on issues of GMO treatment – Bulgarian legislation GM legislation dates back to 1958 Seeds Act. Bulgarian farmers already harvested their first crop of GM herbicide tolerant and pest resistant maize in 1999.

Current trends in Bulgarian and international biotechnological development and the good basis established in the past and rebuilt more recently highlight two broad sectors of biotechnology, which are most relevant for foresight in Bulgaria:

- agriculture (plant and animal biotechnology); and
- beverage and food industry.

Both these areas have been identified by the Government of Bulgaria as priorities in its strategies for long term development and have pledged good financial support by the budget. Additionally the EU has set food safety and consumer protection as corner stones for its R&D activity under the Framework Programmes for Scientific Research. Companies in these branches have been the fastest to recover and are well organized under strong association umbrellas, which would allow for better coordination, cooperation and stakeholder participation in the foresight process.

II. General description of innovation processes in biotechnologies

Biotechnologies belong to the most significant breakthroughs that took place during the last 30 years of 20^{th} c. A paradox of the innovation process in biotechnologies is the rapid development rate of practices outstripping theoretical developments /theoretical reasoning of practical results achieved inclusive/. For example, theoretically zoology is the most advanced science as regards to biotechnology – the studies on developing human genome is practically completed and that of developing the genome of many

animals – almost completed. Studies on developing the plant genome is far from completion. At the same time commercialization of achievements of biotechnology in plants is the fastest growing area of biotechnology after the production of medicines based on fermentation processes and the new methods for vaccine production.

In general, the innovation processes in biotechnology have the following features:

- *Multi-disciplinarity* – the achievements are made by complex, mobile, multi-disciplinary groups of scientists. Due to involving physicians and mathematicians and the use of new generations of computers, the relative price of de-coding the gene sequence in organism has been decreased from USD 2.5 million down to USD 150 per gene from the mid 70s to late 90s of the 20th c.;
- *Commercialization* – it can be seen in the so called fundamental science along with the increase of the role of private investments. Two main issues should be noted here: *Firstly*, there is a new characteristic of the innovation process in biotechnology – obtaining of market value and capitalization of the fundamental science. The development of a certain technology /technological process/ in universities' laboratories usually is followed by the formation of groups of scientists and managers from the private business and by gathering risk capital and public/state grants. The resulting success of such company leads to a great increase in the private funding and finally to listing of the company on the stock market. Many well developed biotechnology companies have gone through this lifecycle. *Secondly*, the development of biotechnology brings very high expectations on the market (sometimes unreal even illusory, yet sometimes completely reasonable). As a result the big private capital starts making efforts to "privatize" the innovation process from its very early stage of lifecycle – that of theoretical development. In its turn early privatization leads to accelerated innovation. Finally, an important feature of the innovation process in biotechnology is the active use of mass media which are an instrument for raising public interest (of both state and private investors);
- *Creation of entirely new barriers to entry in the branch through in-company development of the whole technological process and through creation of new cross-sectoral and multi-company alliances, incl. such with technology consumers and users*. Together with the commercialization of biotechnological achievements in agriculture, emerges the so called system of "full patenting" – from the fundamental development to its commercial implementation. Thus the role of barriers to entry is increased which allows for quick return on investments;
- *International /regional/ and company technology concentration combined with a wide global network (incl. its use*). Biotechnological developments are concentrated in a small group of well-developed countries – USA, Great Britain, Germany, Japan. At the same time in geographical terms the application and usage of technology achievements are much wider due to company globalisation.

A. CHARACTERISTICS OF BIOTECHNOLOGY INNOVATION STRATEGIES – THE NEW IMAGE OF LIFE SCIENCES COMPANIES

- ***New business philosophy*** – the formal start of creating the new image of life sciences companies was the speech of Monsanto's general manager in 1995 that aimed at changing the traditional image of chemical and pharmaceutical companies as companies that pollute the environment and jeopardize the health of population, the cyclic fluctuations of their traditional productions oriented towards recession or slowly developing markets. During the 90s leading chemical and pharmaceutical companies form a new organizational structure thus leaving behind some old inadequate to their new look productions, e.g. traditional chemical productions. This process is called **divestment** and has various organizational forms – from the spin-off of non-specialized productions in different companies (keeping the old shareholders) to the trade sale of specialized company departments to other companies with different

shareholders. In parallel to this they make some sort of "hunting" for crop producing companies as they have the leading role in applying the latest biotechnological achievements;

- ***Biotechnological alliances*** – during the 90s a group of leading chemical-pharmaceutical companies made enormous investments in biotechnology research and developments. In parallel to that new organizational and management structures were created, called **biotechnological alliances**. Their aim is to accelerate the capitalization of certain biotechnological developments /product lines/. The key company – integrator in the biotechnological alliance, is usually an international pharmaceutics or crop-producing company, under the umbrella of which there are specialized biotechnological companies (which receive support from university laboratories), crop-producing companies and their distribution systems, international traders and companies operating in food and drink industry. In some cases other companies are included in the biotechnological alliance – patent and juridical agencies, software producers, investment funds and banks, etc;
- ***Differentiation of agricultural products and changes in the relations with the farmer sector*** – unlike other economy sectors (that already adopted the practice to sell differentiated products under trade mark), even at the end of XX century agriculture offers mass production of standardized products. Implementation of biotechnological achievements allows for product differentiation in many agricultural sectors. The relatively homogeneous market of agricultural products is being segmented down to a market of traditional products and a market of genetically modified products. The market of genetically modified products is further segmented into markets of products with preliminary set features and characteristics. In this relation differentiation should be taken as a process and result from innovation. In many cases differentiation is used as an instrument for capitalization of the company's intellectual property. In other cases it is necessary in terms of consumer and ecological safety. The agricultural sector is composed of hundreds of thousands of farms that are comparatively independent decision makers. A typical feature of the farmer sector (often reasonable) is the considerable dosage of conservatism and certain skepticism to new technologies. From that point of view a key clue to the "access" to new technologies and innovations is the efficiency of biotechnology developments, their commercialization (price) and intellectual property rights in agriculture;

B. ORGANISATION AND STRUCTURE OF THE SCIENTIFIC RESEARCH – CORRELATION BETWEEN PUBLIC AND PRIVATE SECTOR

The major donor for biotechnology science development in the public sector in USA is the Federal Ministry of Agriculture. The funds go to the various universities in the states, where the entire agricultural science is actually concentrated, as well as to the scientific centres of the ministry itself. Besides the funds coming from the state budget, the government provides also the so called tender grants for scientific programmes, scientific teams, and even for individual scientists and researchers. Apart from the Ministry of Agriculture, biotechnology research in the USA is also funded by a number of other federal authorities: National Science Fund, Ministry of Defense, Ministry of Energy, etc. The involvement of the entrepreneur sector in providing funding for agricultural science development is streamed down to providing subsidies to universities and to some private associations.

In European Union countries funding of biotechnology RTD activities is provided both on super-national and national level. In the EU the share of biotechnology research in the total cost of scientific RTD in agriculture is 18-20%. In Bulgaria the funds for research in biotechnology are under the existence minimum, which in practical terms lead even to destruction of some gene-banks.

The RTD various branches' traditional integration is performed by the state universities through their tri-lateral function – to carry out the students' educational process, to carry out RTD activities, and to introduce the results achieved from the RTD into practice. The state universities dispose of scientific and research teams that are in charge of both fundamental and application RTDs and their introduction into practice. The future trend is to maintain and further develop the leading role that universities play in science. But for organizational improvement and expansion of the scientific scale and for

improvement of the correlation between innovations and their end consumers and users certain changes in educational curricula are needed, namely introduction of inter-disciplinary training courses, set out of prizes and awards encouraging joint research programmes, enhancement of relations and communications between scientists and researchers from universities, colleges, laboratories, industrial companies and public scientific centres, encouragement of inter-disciplinary RTD teams by tender-based funds and grants, etc.;

C. CRISIS PHENOMENA IN BIOTECHNOLOGY DEVELOPMENT

In the end of XXc. the biotechnology industry found itself in a quite complicated situation. On the one hand, there is intensive increase of sales of new revolutionary biotechnology products. On the other hand, in the development of a number of biotechnology branches there is an increase of crisis phenomena related mainly to the growing consumer dissatisfaction and concern about food products, as well as green piece organisations' concern.

The reaction of a number of European governments to these concerns lead to the introduction of a moratorium over the import and production of genetically modified foodstuffs in the late 90s. The moratorium decision was passed, even though it was contradictory to an afore-passed decision of the European Commission that had permitted the production of a number of biotechnologically processed products. The legislation and sub-legislative rules and regulations of the EU countries are targeted towards the provision of the so called complete *consumer info-awareness* of the presence of genetically modified components in foods.

That is why a major current problem of the genetic industry as a sub-branch of the economy is that genetically modified foods are not "recognized" on the consumer market as equal to the traditional classic products. This leads to a limited growth potential (and even to a probable future decrease) of the consumer market of genetically modified products as well as to a decrease in the genetically modified goods price level.

D. CONCLUSIONS ABOUT THE BIOTECHNOLOGY INNOVATION PROCESS.

Biotechnologies are a typical example of an innovation cluster process, in which a number of inter-disciplinary RTD teams are involved, diversified fund-raising sources are used (public and private organizations and NGOs), and fundamental scientific discoveries are "privatized" by the private capital in their earliest stage of development.

The innovation process in biotechnologies lead to structural modification of all sub-branches, directly or inderactly related to biotechnologies, and to the set-up of big companies and global alliances of companies targeting the commercialisaation of scientific achievements. The commercial assimilation of biotechnology achievements is possible due to and only within international cooperation.

The innovation process goes in parallel with the search and introduction of new economic mechanisms for market players' encouragement within the vertical product chain. The constantly growing role of the protection system of intellectual property rights and "full patenting" is worth noting, thus acting as a new barrier to the invasion of biotechnology achievements into the sector and a new means of profitable "exploitation" of biotechnology achievements.

The role of the state as a guarantee for protection of contemporary market institutions (those for intellectual property rights inclusive), for consumer rights protection regarding the products of the new branches, and for environment protection is increasing. Along with that, the respective state and international regulations remain yet not quite efficient and that is why they are in the process of constant modification.

In conclusion, the basis that Bulgaria established and developed in the 70s and 80s in terms of education, research and innovation in biotechnology gives the ground to include this sector in the

forecoming technology and innovation foresight, the more so as this is in line with Bulgaria's future integration into the EU.

III. Traditional Bulgarian biotechnology-intensive industries

Bulgaria is a country with old traditions in the classical fermentation technologies like brewery, wine and milk industries. It is an old but still preserved tradition for Bulgarians to make their own home-made wine and spirits.

A. WINE PRODUCTION

Until 1947 more than 20 000 small Bulgarian private companies had produced and sold wine in Bulgaria and Europe. The first big wine company (wine cellar) has been founded in Suhindol in 1909. After nationalisation (1947) many big wine factories and plants have been built in Sofia, Pleven, Russe, Lyaskovetz, Targovishte, Preslav, Pavlikeni, Pomorie, Burgas, Sliven, Chirpan, Perushtica, Assenovgrad, etc. Grape collection and processing have been modernised and discontinuous fermentation was soon introduced. The yield of wine (related to grape) increased from 40-50% for the home-made wine to over 73% for the industrially produced one. Wine production was standardised and Bulgarian wines became well known to the world market. The most popular wine trade marks are Misket, Muskat, Cabernet, Mavrud, Gamza, Dimiat, Kadarka, etc.

B. BREWERY

Brewery is an old industry too. The first Beer Cartel was founded in Sofia in 1908. It included 7 beer factories in Sofia, Lom, Plovdiv and Shoumen. A new Cartel (called United Breweries) was founded in 1927 and included 12 beer factories.

C. DAIRY INDUSTRY

One of the oldest Bulgarian fermentation products is the "sour milk" (incorrectly translated "yoghurt"). It is a unique milk product, different from the world known yoghurt. Unlike yoghurt, Bulgarian sour milk is made of two bacteria *(Lacobacillus bulgaricus* and *Streptomyces termophylu*s) living in symbiosis. Each one of the two microorganisms produces different biologically active substances. Maintenance of this symbiosis requires a special milk composition. This unique composition is typical for the Balkan countries milk only. That is why it is not easy to organize a stable production of real sour milk in countries outside the Balkans. The classical yoghurt is a monomicrobial product. Other traditional Bulgarian milk products (typical products of the enzyme technology) are the "white cheese" (incorrectly translated "feta cheese") and the "kashkaval" (yellow cheese).

D. ANTIBIOTIC PRODUCTION

The fist plant for manufacturing of antibiotics was built in Bulgaria (Razgrad) in 1952-1954 and the second one (in Peshtera) in 1959-1960. They both used to produce (until 1989) more than 25 different antibiotics and 80% of their production was exported. About the same time several pilot plants and plants for fodder yeast have been also opened.

E. LEACHING

The first industrial installation for underground bacterial leaching was opened at the mine "Vlaikov Vrach) in 1970.

In order to satisfy the needs of Bulgarian fermentation technology for technological improvements, new technologies and qualified human resources, many specialized research institutes and departments to the universities were founded between 1950 and 1960. Some of them were: Higher Institute for Food and Flavour Industry (Plovdiv), Research Institute of Wine Industry (Sofia), Research Institute of Wine Industry (Pleven), Research Institute of Brewery (Sofia), Research Institute of Milk Industry (Sofia), Research Institute for Antibiotics (Razgrad), Research Chemical-Pharmaceutical Institute (Sofia), etc.

IV. Historical review of biotechnology in Bulgaria

A. THE UPSURGE (1983-1989)

Biotechnology became a priority area for the economy of the former Eastern Block countries, including Bulgaria, after 1983. It was regulated by a special *Programme for Development of Biotechnology in Bulgaria*, which was controlled by the former State Committee for Science and Technology and the Central Committee of the Communist Party. Hundreds of millions of dollars were invested during the above period to ensure development of biotechnology in the following areas:

- Fermentation technology
- Pharmaceutical biotechnology
- Plant biotechnology
- Environmental biotechnology
- Research and development (R&D)
- Education

1. Fermentation biotechnology

Fermentation biotechnology (industrial scale) was developed in the two plants for antibiotics in Razgrad and Peshtera and in the plant for manufacturing of enzymes in Botevgrad. These three plants were renovated and expanded and many other small pilot-plants and semi-industrial laboratories were created for production of: **antibiotics for human and veterinary medicine;**
odder yeast and alga; baker yeast; pesticides; citric acid; itaconic acid; gluconic acid; lactic acid; lyophilized *Lactobacillus bulgaricum*; vinegar; ethanol; decstran, etc.

2. Pharmaceutical biotechnology

Pharmaceutical biotechnology was based on the former pharmaceutical giant "Pharmachim" with its five main branches in Sofia, Razgrad, Peshtera, Stanke Dimitrov (now Dupnitza) and Troian. Besides Pharmachim, several small factories and pharmaceutical pilot-plants were founded for production of: **specific proteins, enzymes and hormones; vitamins, vaccines and antiserum for human and veterinary medicine; biostimulants; biosensors, etc**.

3. Plant Biotechnology

For the last 20 years 95% of the arable land in Bulgaria for the most important agricultural crops was planted with cultivars developed by local breeding institutes. The latter have also produced varieties which have been proved to be most successful in the CIS countries, Romania, Greece, Yugoslavia, Slovenia, Croatia, France, etc.
In order to strengthen crop breeding in Bulgaria a National Programme for Plant Biotechnology was launched in 1983. As a consequence in 1985 the Institute of Genetic Engineering (IGE) (at present AgroBioInstitute - ABI) was established at the Agricultural Academy with the intention to become a National Center for Plant Cell and Molecular Genetics Research that is responsible for co-ordination of plant biotechnology activities in Bulgaria and for the cooperation with foreign research centers, universities and companies in that field.

The Institute was recognized as a part of the National Plant Biotech Network (table 1) and as a result the first scientific events in plant biotechnology were organized in Bulgaria. Serving as national Center of the Plant Biotech, IGE established close contacts with breeding institutes and a whole network of specialized laboratories – tissue culture, micropropagation and immunodiagnostics. The distribution of expertise and training of scientists from other Bulgarian institutions became a common practice.

Table 1. National Plant Biotech Network

	ABI (IGE) - Kostinbrod	
I. Methodological laboratories	***II. Tissue culture laboratories in Bulgarian breeding institutes***	***III. Scientific – applied laboratories***
⇓	⇓	⇓
1. Doubroudja Institute of Agriculture, G. Toshevo	1. **Institute of Horticulture and Canned Foods,** Plovdiv	**1. ABI, Dept. of flowers,** Negovan
2. Institute of Genetics, Sofia	2. **Institute of Fruticulture**, Plovdiv	**2. Institute of Fruticulture** Plovdiv
3. Institute of Plant and Genetic Resources, Sadovo	**3. Institute of Agriculture,** Shumen	**3. Institute of Horticulture and Canned Foods,** Plovdiv
	4. Institute of Agriculture, Kjustendil	
	5. Institute of Viticulture and Vine Production, Pleven	
	6. Institute of Forage Crops, Pleven	
	7. Institute of Maize, Kneja	
	8. Institute of Mountain Animal Husbandry and Agriculture, Trojan	
	9. Institute of Plant Protection, Kostinbrod	
	10. Institute of Agriculture and Seed Science "Obraszov chiflik", Russe	

4. Environmental biotechnology

Environmental biotechnology dealt with water purification; soil cleaning (bioremedation); leaching (biometallurgy).

The ***R&D*** of the *Programme for Development of Biotechnology* in Bulgaria was the most impressive one. Tenths of the existing Bulgarian universities and research institutes has been affiliated to this programme and many other new centers, institutes, departments, research laboratories and groups were founded all over the country to work on different fields of biotechnology. Although R&D was spread over all areas of biotechnology, a special priority was given to the genetic and cell engineering.

Millions of US dollars were supplied to relevant organizations for equipment, consumables and staff training. More than 1500 different projects were subsidised by the Programme during 1984-1989 and tenths of original technologies were developed and patented in Bulgaria and abroad.

The *Programme for Development of Biotechnology* in Bulgaria aimed also to create a modern biotech park (called National Center for High Biotechnology) at Gorni Lozen (near Sofia). The NCHB was designed to combine both R&D and manufacturing of high biotech products and should accommodate 1500 employees. Due to financial reasons, the NCHB had never been built.

The ***education*** in biotechnology was organised at both graduate and postgraduate levels. In order to satisfy the foreseen needs of qualified personnel for the future years, a special *Center of Biotechnology*

for education in biotechnology at MS level had been created as an interuniversity unit between the three universities: Sofia University (Faculty of Biology), University of Chemical Technology and Metallurgy (Sofia) and the Technical University (Sofia). A pilot programme was launched for the education in this center according to which the students were trained all together during the first three years. After that they were split into three streams for further (two year) education in one of the three partner universities.
Finally, they had to defend a master thesis. A great number of theses were worked out in the institutes of Bulgarian Academy of Sciences such as the Institute of Molecular Biology, Institute of Microbiology, Institute of Immunology, etc. They also used to accommodate tenths of Ph.D. students and junior university teachers for postgraduate and post-doctoral training. Several hundreds of biotechnologists succeeded to obtain their MS and Ph.D. degrees during the years 1984 and 1990. Most of them remained jobless and were forced to leave the country.

B. THE CRISIS (1989-1997)

During this period the country as well as all the institutions were subjected to a completely new situation with the establishment of market economy, changes in all legislation, organization and financial support for the science. The state funding for the institutions decreased drastically. Despite all the problems, some research institutes (for instance Institute of Genetic Engineering, Kostinbrod) were able to maintain high level of research and to increase their international contacts and collaboration. The institute jointed a unique form of collaboration – the Norman Borlaug Institute for plant science Research, together with colleagues from SDe Montfort University, Leicester, UK, the Institute of Experimental Botany, Prague, Czech Republic and the Institute of Genetics, Beijing, China.

In general the products of Bulgarian biotechnology were designed for domestic use and the markets of the former Eastern Block countries. They did not satisfy the world accepted GMP requirements and therefore were not eligible for export to the western market. That is why the collapse of the Former Socialistic Block and the Soviet Union in particular had a crucial effect on Bulgarian biotechnology. The latter was stricken also by the sharp transition from a state controlled to free market economy. The lack of market had a catastrophic effect on the Bulgarian biotech enterprises.
The most significant changes that happened to biotechnology in Bulgaria after 1989 were the following:

- The State Committee of Science and Technology, National Centre of Biotechnology, educational Center of Biotechnology, etc., were immediately closed and biotechnology was not longer a priority area for Bulgarian economy.
- A great number of small enterprises collapsed and the biggest ones (the antibiotic plants in Razgrad and Peshtera, the plant for enzymes in Botevgrad, etc.) restricted their activity to the state of survival.
- Many research institutes and universities had either stopped or drastically reduced their R&D activity.
- Hundreds of specialists (MS and Ph.D.) trained in biotechnology remained unemployed and left the country.
- Expensive equipment designed for research and industrial purposes had been abandoned and was either stolen or severely damaged.

The apocalyptic stage and agony for Bulgarian biotechnology was additionally extended until recently because of the slow privatisation and unstable economy.

C. THE REVIVAL (1997-2000)

Economical conditions in Bulgaria were gradually improving during that peroid, which favoured the development of Bulgarian biotechnology. In these years a stable and sustainable growing economy was established in Bulgaria, mainly due to the implementation of the Currency Board. In spite of the positive changes in the national economy, the strong budget restrictions led to drastic reduction of the state funding for scientific organizations, especially to those belonging to Agricultural Academy (now National Centre for Agricultural Science, NCAS).

The continuing insufficient financial support from the government forced the Research Institutes and Universities to search very actively alternative source of funding – both international and national collaboration and projects, for instance the Framework Programmes of the European Community.
Some of the old biotech enterprises re-initiated their activities on credits and others were privatised and re-established as new companies. The R&D in universities and research institutes was re-activated on account of foreign grants and international research programmes. Educational system was reorganised to fit the western standards and the education in biotechnology was re-established in several universities, although in different forms (see below).

1. Pharmaceutical and Fermentation Biotechnology

Following 1989 the former pharmaceutical company Pharmachim was split into several independent companies. They all were privatised and some of them were reunited under the new-born holding **"Balkanpharm**a". Biotech products are presently manufactured by the company **"Balkanpharma-Razgra**d" (the former antibiotic plant in Razgrad). Its present product list is much shorter than before and includes two categories of products: 1) Substances and 2) Pharmaceutical formulations.
The list of substances includes:

- **Tilosin**
- **Apramycin**
- **Tobramycin**
- **Sulbactam**

The list of final (ready-made) pharmaceuticals consists of **93 items** (not presented here) which are based on both own substances and imported antibiotics.
Presently Balkanpharma-Razgrad employs 20-30% of its technological capacity. Most of the old technologies are now frozen because of necessity of technical renovation and technological improvements to satisfy the GMP requirements. The R&D activity at Balkanpharma-Razgrad is carried out in the Research Institute for Antibiotics (belonging to the same company). It is focused on the development of new technologies for production antibiotics and new final forms.

"Biovet" is an independent private company based on the former branch of Pharmachim in Peshtera. It is specialised in manufacturing pharmaceuticals and other products for veterinary and farming. The product list of Biovet includes a great number of final forms classified into the following five categories of products:

- ***Tylosin* product***s: Tylovet T (tylosin tartarate), Tylovet P (tulosin phosphate), Tylovet P (tylosine base), Tylovet 10% and 25% (injection form of tylosin), Tylovet pulvis, Tylovet forte (tylosin plus bromhexin), Bromhexotylosin for poultry, Bromhexotylosin for pigs, etc.
- ***Antiparasitic preparation***s: Bulmectin (containing abamectin), Pandex (ivermectin), Abantel, Prazimec C and D, etc.
- ***Anticoccidial***s: Salinopharm (salinomycin), Monensin, Yumamycin (maduramycin).
- ***Nutritional food additive***s: Pharmastim (flavophospholipol), VAM-F for pigs (flavophospholipol, vitamins and minerals), VAM-F for poultry, VAM-F for calves and lambs, VAC-22F, etc.
- ***Dietary supplement***s: Biomix for pigs, for fish, for poultry and rabbits, for horses, for sheep and lambs, for calves and cows, Vitamin C 10% (injection), Enterosan *(Lactobacillus bulgaricus &Lactobacillus LAB*8).

The R&D at Biovet is carried out in its well equipped research laboratories which are specialized in the following areas:

- Chemistry
- Microbiology & Biochemistry
- Analysis
- Final forms

The production facilities of Biovet are GMP certified and its products comply with the European and British Pharmacopoeia standards.

"DeoDan" is a small private company organized on the basis of the former Research Institute for Anticancer Antibiotics in Sofia. Its activity includes development and manufacturing of:

- ***Biologically active substances and components***
- ***Final Products on their basis***
- ***Pharmaceuticals for human medicine and veterinary***
- ***Dietetics***
- ***Cosmetics***
- ***Health food products***

Most of the DeoDan products are based on extracts and derivatives of the bacterium *Lactobacillus bulgarius* and on their own (patented) strain *Lactobacillus tumoronecroticance B5*1. The most popular of the DeoDan products are:

- Deodan for. i.v. application (a preparation with tumor narcotising activity)
- Deodan cream (for skin burns, slowly closing wounds and varicose ulcers, cicatrices after burns and injures)
- Night and day cosmetic creams, milks and lotions
- Anti-acne cream
- Gastropharm (for treatment gastritis and ulcers)
- Normoflor (for intestinal flora normalisation after extensive antibiotic treatment)
- Yoghurt (prepared with the strain *Lactobacillus tumoronecroticance B5*1)
- Ice cream from soy yoghurt (prepared with the above strain)
- Solacta (a dietetic drink based on fermented soy milk).

"National Center for Infectious and Parasitic Diseases" (NCIPD) in Sofia owns research laboratories and a pilot plant for production of the following products for human application:

- vaccines
- antisera
- blood products

Most of the NCIPD products are designed for the domestic market.

"Plastchim" is a private company based on the former plant for enzyme preparations in Botevgrad. Its product list includes the following products:

- **Enzymes**

- Amylases (from *Bacillus subtilis, Aspergillus orizae* and *Aspergillus niger*)
- Proteinases (alkaline proteinase from *Bacillus subtilis*, neutral proteinase from *Bacillus mesentericus*, acid proteinase from *Aspergillus niger*)
- Cellulases (Xilanase from *Aspergillus niger*, Cease from *T. viridae*)
- Pectinases (from *Aspergillus niger*, macerase from *Aspergillus niger*)
- Lipases (from *Rhizopus arrhizus*)
- Oxyreductases (lipooxigenase from *Penecillium sp*.)

- **Enzyme complexes**

- Pivosin (for brewery)
- Aprozyme (for textile, leather and detergent industry)
- Protisine (food additive for calves and cows)
- Stabilin (for the wine industry)
- Celuten (for textile and washing)
- Avapan (bread additives)

- **Bioactive substances for human and veterinary medicine**

- Gastropharm (for treatment of gastritis and ulcer)
- Normoflor (for treatment of colitis, diarrhea, proctitis, etc.)

- Biolact 65 (biostimulant enhancing recovery of sportsmen)
- Vitasan P (prophylactic of arteriosclerosis)
- Faringolact (for treatment of tonsillitis, laryngitis, etc.)
- Hepatosan (for treatment of liver diseases)

Plastchim does not have GMP conditions and its products are addressed to the domestic market and as well to the markets of some former Eastern Block countries.

The ELBY Company is an undoubted leader in scientific and technology developments of sour milk (Bulgarian yogurt). A joint scientific research project of the ELBY Company and Japanese scientists has been successfully completed in this area. The company has also developed a number of specialized dairy products by lactic acid fermentation for recovering, containing only Lactobacillus LBL4; sour milk for drinking obtained through fermentation of symbiotic starters, various diet kinds of Bulgarian yogurt, etc.

2. Dairy products obtained by lactic acid fermentation and bio-active substances

An increase of the biological value of theBulgarian yogurt can be reached using some starters of *Lb. bulgaricus* and *Str. Thermophilus*, wchich are capable to release functional peptides and synthesize biologically active compounds. In the recent years intensive researches on the presence and action of bioactive peptides in Bulgarian yogurt have been done. Bulgarian scientists and technologists have taken active part in those researches. An increased interest in such bioproducts was registered. These peptides are obtained in decomposition of casein during the lactic acid fermentation and they influence blood pressure. It has been proved that these peptides not only decrease the upper and lower values of blood pressure, but they also prevent their sharp increase.

3. Lactic acid fermentation on non-dairy substrates

In the recent 20 years lots of experiments have been made worldwide, in Japan especially, for cultivation of lactic acid microorganisms, *Lactobacillus bulgaricus* and *Streptococus* thermophilusc in particular, on non-dairy substrates: soy-bean products, peanut milk, wheat germs, etc. In the early 90s Bulgarian BAS scientists together with the ELBY Company developed a patented technology for fermented grain foodstuffs based on oats and Bulgarian yogurt symbiotic starter.

Recently the UFT – Plovdiv has developed a technology for obtaining a wholesome oats kind of drink, fermented together with other lactic acids microorganisms.

The probiotics are a new area that is widely involved in the production process of the ELBY Company and is of great significance to the leading biotech companies in Bulgaria. The probiotics are a new class of antimicrobialk preparations, they are composed of live microorganisms in a liophilic or non-dried condition. They can be obtained from non-pathogenic and lactic acid fermentation bacteria that have been extracted from the digestion system of healthy animals. The currently produced probiotics contain primarily the following: *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii, ssp. вulgaricus, Streptomyces salivarius, ssp. thermophilus, Enteroceus faecalis, E.coli*

4. Mineral and Environmental Biotechnology

Mineral and Environmental Biotechnology deals with bacterial leaching of heavy metals (copper, gold, uranium, etc.) from poor ores, microbial removal of certain undesired compounds from ores and minerals, bioremedation (water and soil cleaning by microbiological methods), production of petrol products from poor sources, gas production from farm dumps (biogas), etc.

The following plants and pilot plants are (or have been) functioning for:

- ***Bacterial leaching*** of **copper** from poor or unbalanced ores: "Tzar Assen" (Panagyurski Medni Mini), "Assarel" (Stara Planina), "Medet" (Panagyurishte).
- ***Underground bacterial leachin***g: Two industrial installations (the biggest in Europe) have been functioning between 1970-1999 at the mine "Vlaikov Vrah".
- ***Bacterial leaching*** of **uraniu**m:

- One industrial installation at Simitly (1985-1990)
- One pilot plant in Momino.

- ***Bacterial leaching*** of **gold:** Two pilot plants (1995-1998) at Panagyurski Medni Mini.
- ***Production of petrol product***s: One pilot plant (1995-1998) at Tyulenovo.
- ***Bioremedatio***n:

- For cleaning of industrial waste water: One industrial installation (1988-1999) at Tyulenovo and Dolni Dabnik and one pilot plant at Burgaski Medni Mini for cleaning of waste water from the mining industry.
- One installation for purification of water contaminated by ammonium

- ***Removal of iron*** from quartz sand and kaolin: one pilot plant (1984-1987) at the Senovo mines.
- ***Bioconversion of waste materials*** from the mining industry: One pilot plant at Panagyurishte.
- ***Water cleanin***g: many modern water plants supplying drinking water to the big cities.

5. Genetic Engineering of microorganisms

The only one Bulgarian company dedicated to the application of recombinant DNA technology is **"Pharmage**n". It is a small private company owing patents (including European), bacterial strains and know-how for production of recombinant human interferons alpha 1 (hIFN-a1) and gamma (hIFN-g) as well as of several pharmaceuticals based on the hIFN-g. The Pharmagen product list includes the following final forms:

- Gammaferon inj. (injection form of gamma interferon)
- Gammaferon eye drops (for treatment of viral eye diseases)
- Virogel G (gel for treatment of viral skin diseases)
- Ribovasan (gel for treatment of skin burns and slowly closing wounds)

Although these preparations have shown excellent results in clinics and are allowed for sale in Bulgaria, they are not on the shelves because Pharmagen does not have any industrial capacity and needs partners and investors.

6. R&D in the Field of Biotechnology

The R&D in biotechnology in Bulgaria is carried out in the following research institutions listed below:

- Biotechnology field: Ffermentation, enzyme and pharmaceutical biotechnology.
  - Higher Institute for Food and Flavour Industries (Plovdiv) - research related with wine and beer production; selection of new yeast strains with improved technological characteristics; identification of new antibiotics; selection of *Streptomyces* strains having improved productivity of antibiotics; development of new technologies for production of aminoacids, vitamins and food additives; reseach on *Lactobacillus bulgaricus*; research on enzymes related with milk, bread and other products.
  - Research Institute for Antibiotics (Razgrad) - selection of antibiotic producers with improved productivity and technological advantages; improvement of technologies for fermentation of *Streptomyces* and purification of antibiotics.
  - Research Institute for wine industry (Sofia) – development of new approaches for stabilization of wines and new methods for analysis; development of technologies for isolation of biologically active substances from wine waste materials (seeds and peels).

- Department of Biotechnology (at the University of Chem. Technology Sofia) – biosensors; immobilization of enzymes (enzyme bioreactors); bioconversion of farm dump materials (biogas production).

- Biotechnology field: Mineral and environmental biotechnology.
  - Department of Engineering Geoecology – bacterial leaching of heavy metals from poor ores; removal of heavy metals from ores and minerals; purification of waste waters to drinking water quality; microbial removal of H2S from natural gas; detoxification of agricultural lands polluted by heavy metals; microbial cleaning of oil polluted soils.
- Biotechnology field: Genetic engineering.
  - Institute of Molecular Biology (BAS) – construction of recombinant bacteria for production of biologically active proteins for medical use (interferons, calcitonin, antiviral proteins, etc.); research on improvement of gene expression; stabilasation of bacterial plasmids; structure-function relationship studies; development of technologies for purification of recombinant products and new pharmaceuticals.
  - Institute of Genetic Engineering (Kostinbrod) – laboratory and field tests with transgenic plants (tobacco, potato, etc.) created by Monsanto, Astra Zeneca, etc.; transformation of vine and other plants with specific genes;
  - Department of Plant Physiology Faculty of Biology (University of Plovdiv) – expression of foreign genes in cultivated plants (tobacco, maze, etc)
- Biotechnology field: Biotechniques.
  - Department of Biotechniques (Technical University Sofia) – biosensors for registration and quantitative analysis of certain biologically active and important substances.

7. Bioengineering

The role and accents in bioengineering streams in science and industry are changed, the rate of integration among different engineering sciences, microbiology, informatics and others is enlarged, e.g.:

- automatisation of projection in bio-machine building, information technologies are used for archiving and multiplication of engineering developments in other machines or reactors for biotechnology;
- bioinformatics is called "the interface between humanitarian and computer sciences", "intersection between humanitarian sciences and informatics", "computing biology";
- Genetic engineering offers new opportunities to the contemporary medicine through problem solving in prophylaxis, prevention and medical treatment of many innate, chronic and degenerative diseases, the genetic surgery and other similar scientific streams appear on one hand, on the other – genetic engineering develops new technologies for obtaining of exceptionally important for the medical practice albumens and agents, such as insulin, interferon, growth hormones and others;
- Immune biotechnology is related to the production of new vaccines for prophylaxis of diseases in human medicine and stockbreeding; production of monoclonal antibodies, different reagents used in the diagnostics of възбудители of diseases, as well as for cleaning of vitamins, different albumens (enzymes, toxins, etc).

As a result of this the worldwide opinion is that contemporary Biotechnology has a polyvalence character and the development of any of its branches is impossible without the development of the others, including the related bioengineering sciences.
New trends in the development of biotechnologies and the related bioengineering sciences are connected to their sphere of application which for Bulgaria is as follows:

1. Agriculture and stockbreeding;
2. Industry (food, drink, pharmaceutics);
3. Medicine (new materials, medicine and diagnostic materials, personal systems for diagnostics, treatment and dozing medicines);

4. Ecology (monitoring, short- and long-term forecasting of pollution, informing, purification);
5. Science and Education (secondary education, higher education – bachelor's and master's programmes, doctor programmes; programmes for prequalification or enhancing qualification)

Some of the new trends in the development of biotechnology are as follows:

- Development of machines, bioreactors and others for research and industry, for agriculture, stockbreeding and other branches of industry;
- Development of systems for automatised design of bioreactors and other machines (because of the economical efficiency of the equipment on the basis of analysis of existing solutions, with the use of internet in an environment of globalization);
- Development of database and knowledge bank with engineering projects and solutions in the field of systems for machines' design with the purposes of using past experience (defending the intellectual property – technical documentation, schemes, drafts of machine components, components, photos, graphics, technical protocols, etc.);
- Increase in efficiency in the production of spare parts (on the basis of information kept in the database, when required by clients, regardless of the expiry period of machine equipment);
- Development of methods and sources for economic analysis and forecast of the quality and the economical efficiency of the equipment of bioprocesses.

V. Education in Biotechnology

Reorganization of Bulgarian educational system has started several years ago in order to introduce a **three level university system** ending up with **BS** (bachelor), **MS** (master) and **Ph.D.** respectively. The three university degrees in biotechnology can be obtained in several state universities (see below). The BS degree was introduced several years ago after a joint Tempus project (entitled ***"National Curricula and Study Programmes for Bachelor Degree in Biotechnology***") had been run within three Bulgarian universities (University of Chemical Technology and Metallurgy, Higher Institute for Food and Flavour Industry in Plovdiv and the University of Plovdi**v**), the Institute of Molecular Biology and four European universities. Bulgarian institutions presently preparing specialists in the field of Biotechnology are the
following:

- ***Sofia University*** (Faculty of Biology): Annual enrollment - 30 students. Until now all students graduated with a MS degree (following the old one level system). Since 2001 they will end up with a BS degree and about 20% of them are expected to join the next MS and/or Ph.D. programmes.
- ***University of Chemical Technology and Metallurgy*** in Sofia (Department of Biotechnology): Annual enrolment - 25 students. Their status is as above.
- ***Higher Institute for Food and Flavour Industry*** in Plovdiv (Department of Biotechnology): Annual enrolment - 35 students. Their status is as above.
- ***University of Mining and Geology*** (Department of Engineering Geoecology): Prepares few MS and several Ph.D. students in the field of mineral and environmental biotechnology.
- ***Plovdiv University*** (Faculty of Biology): Prepares several MS and Ph.D. students in the field of plant genetic engineering.
- ***Institute of Molecular Biology*** (Bulgarian Academy of Sciences): Prepares Ph.D. students in bacterial and pharmaceutical genetic engineering.
- ***Institute of Microbiology*** (Bulgarian Academy of Sciences): Prepares Ph.D. students in fermentation biotechnology.

There are also two colleges preparing technicians for the needs of Bulgarian biotechnology:

- ***College for Biotechnology*** (at the University of Chemical Technology and Metallurgy in Sofia): Annual enrollment - 20 students.

➢ ***College for Biotechnology*** in Razgrad (at the University of Russe): Annual enrollment - 20 students.

Besides the above specialists, Bulgarian biotechnology relies also on chemists, biologists, food technologists, pharmacists, etc., graduated from one of the above or other Bulgarian universities.

VI. New trends in the development of Bioengineering sciences and RTD

The new trends in the development of biotechnologies and bioengineering sciences are close related to their areas of application in Bulgaria:
- agriculture
- food and drinks industries, pharmaceuticala industry
- ecology (monitoring and forecast of level of pollution; purification)
- science and education

Some of the new trends in the development of biotechnologies and bioengineering sciences are given below:

A. BIOAUTOMATION (AUTOMATION OF BIOPROCESSES)

- Development of hardware and software biosensor systems with high precision and sensitivity, for determination of hard-to-measure or dangerous biotechnological parameters;
- Development of scientific research automation systems; automation of the research activity
- Development of systems for industrial bioprocesses automation
- Development of methods and tools for economic analysis and prognosis of bioprocesses' quality and economic efficiency; introduction of software systems for prognosis of the final composition and quality of commercial bio-products – particularly in Food and Beverage Industries; binding with Internet, for effective usage of World and Bulgarian experience;
- Development of specialized systems for ecological monitoring, for announcement and implementation of purifying actions against water, air and soil pollution with bio-products;
- Development of simulative software systems for training and prequalification of staff for the biotechnology.

B. BIOINFORMATICS

- Elaboration of a common programme for development, application and training in bioinformatics in Bulgaria, in line with the perspectives for integration in NATO and the European Community; development of an education curricula and plans for the qualification of specialists in bioinformatics, in partnership with academic partners from BAS and the universities;
- Servicing Bulgarian and foreign industrial and scientific organizations by providing results and outcomes from bioinformatics development programmes;
- Compilation and maintenance of data-bases and knowledge-bases with biological information (genetic information inclusive) for scientific and industrial purposes (using pf Pearl, Java under Unix; adaptation for using under Windows and C++);
- Development, testing and application of automation systems and new mathematical methods (classical, artificial intelligence, knowledge-based modeling and others) for prediction of biological and genetic phenomena in cells and molecules, based on data samples, their mathematical transformation and manipulation;
- Developing of precise mathematical methods and software tools for the objectives of molecular biology, functional genomics (with the aim predetermine the biological functions of genes and genetic products, according to their primal sequence and structure, rapid

detection of transcription factors in co-regulated genes), for prediction and fast comparison of protein structure and DNA;.

- Application of existing systems for ecological purity testing of imported and domestic foodstuffs and drinks.

VII. Intellectual Property and Legislation Related with Biosafety

Since 1993 Bulgaria has a new intellectual property law and a new patent system. It replaced the former "Certificate for Invention" system and is closer to the European rather than to the American patent system. During this transition a great number of inventions in biotechnology have not been converted into patents and therefore are not valid anymore. The reasons for this were the financial inability of inventors to maintain their own patents and the dramatic decline of biotechnology at that time and therefore the lack of interest on the side of enterprises towards technological innovations.
In 1994 a Biosafety Committee was created in Bulgaria to elaborate rules and precaution measures for applying genetic engineering methods and use of genetically modified organisms (GMOs) in Bulgaria. In February 1996 the Ministry of Agriculture has established a Council to control the use of GMOs. This year (2003) a draft of a Law for GMOs is worked out and presented to the Parliament. It is similar to the laws of other European countries and concerns the rules, licensing and sanctions related to the use and release of GMOs. It is anticipated that the Law for GMOs will be accepted in 2003.

VIII. Classical Technologies Related to Biotechnology and Applied in Bulgaria

As already mentioned, Bulgaria has old traditions in some classical technologies related to biotechnology and they were among the first showing a remarkable progress in the time of free market economy. Most promising of them are the following:

- Wine industry
- Brewery
- Meat processing industry
- Milk processing industry
- Grain processing industry
- Essential oil and fragrance industry
- Pharmaceutical industry based on natural products

The contemporary biotechnology methods hold essential significance in the agriculture of ***natural*** plants and animals, even that is insufficient appreciated. Some important to Bulgaria perspectives for an increased production efficiency keeping the country's world recognition as producer of ecological production will be covered in the current document.

It could be claimed that in the food and beverage industry there is the most restricted possibility to apply genetically manipulated organisms (GMO). The exact way for modernization of the present food and beverage industry is the application of improved microbial and enzyme technologies.

In general, the microbial strains (starter cultures) for industrial purposes are kept in the National Bank for Industrial Microorganisms and Cell Cultures. The National Bank was established in 1983 - the Bank was recognized as international in 1987. It is authorized to receive and preserve microorganisms and cell culture used for scientific research, industrial production and diagnosis purposes. Subject to preservation are bacteria and actinomycetes, fungi and yeasts, viruses and cellular culture. Pathogenic microorganisms are not accepted. Over 3500 kinds of cultures are kept in the bank for scientific, educational, diagnostic and industrial purposes.
In the recent years most bread, milk, meat and canned food producers use starter cultures for the biotechnology process of food products manufacture. Leader companies in the production of beer (Zagorka, Kamenitsa) and dairy products (Danone, Elby, Milky Way) maintain and develop specific strains for the manufacture of products with permanent high quality. Local collections of

microorganisms for application in biotechnology are maintained in the institutes of Bulgarian Academy of Sciences (BAS) and National Centre for Agricultural Sciences as well as in the respective microbiology university departments.
The general areas of microbial and enzyme technology application in the food and beverage industries are given below. These major areas of biotechnology methods application for production of food additives demonstrate the great potential and capacity of Bulgarian companies operating in this sector. These companies produce 40% of the daily consumprion of foodstuffs by the Bulgarian population. The main application areas of microbial and enzyme technology in the food and beverage industries are as follows:

- Food and beverage industries:
  - ✓ Dairy industry – lactic acid microorganisms, brine white and yellow cheeses, delicious cheeses, whey products;
  - ✓ Grain, bread and sugar – bread yeasts, additives for bread production, invert sugar, glucose fructose syrups, boza;
  - ✓ Beverage industry – yeasts, enzymes, beer, wine, spirits, vinegar;
  - ✓ Special foods and additives – recovering foods for sportsmen, food additives; functional foods; foods for adult population;
  - ✓ Canning industry – juices production; production of foods from legumes seeds with reduced content of α-galactosides;
  - ✓ Wastes treatment.
- Agriculture:
  - ✓ aquacultures,
  - ✓ livestock breeding,
  - ✓ plant growing.

A. FOOD AND BEVERAGE INDUSTRIES

1. Dairy industry

a) White and Yellow Cheese

The white brine cheese is one of the most widely consumed food produced in Bulgaria, there is as well a real potential for increase of its export. That is a typical combination of enzyme and microbial technologies and our country has long traditions in production and science in this area. Biotechnology methods are applied in the production of various kinds of yellow cheese and here the country has got great potential as well. The major part of the currently used enzymes (protease, lipase, lactase etc.) is imported.

b) Dairy products obtained by lactic acid fermentation

Our country is famous for its production and great consumption of Bulgarian yogurt that is produced by using symbiotic starters of *Lactobacillus bulgaricus* and *Streptococus thermophilus*. A typical peculiarity of the classic product is the domination of bacillus over cocci in the end product. In the recent years the production has been made up-to-date in a number of companies, new equipment was introduced and now the hygiene requirements are met. In scientific terms research has been carried out on genetic classification by type and on identification passport of the starter strains. This research must continue in terms of the genetic determination of their useful features.

The ELBY Company is an undoubted leader in scientific and technology developments of Bulgarian yogurt. A joint scientific research project of the ELBY Company and Japanese scientists has been successfully completed in this area. The company has also developed a number of specialized dairy) products by lactic acid fermentation for recovering, containing only Lactobacillus LBL4; yogurt for

drinking obtained through fermentation of symbiotic starters, various diet kinds of yogurt, etc. An on-going joint project with AgroBioInstitute is developing DNA markers for protection of the unique strains

c) Dairy products obtained by lactic acid fermentation and bio-active substances

An increase of the biological value of theBulgarian yogurt can be reached using some starters of *Lb. bulgaricus* and *Str. Thermophilus*, wchich are capable to release functional peptides and synthesize biologically active compounds. In the recent years intensive researches on the presence and action of bioactive peptides in Bulgarian yogurt have been done. Bulgarian scientists and technologists have taken active part in those researches. An increased interest in such bioproducts was registered. These peptides are obtained in decomposition of casein during the lactic acid fermentation and they influence blood pressure. It has been proved that these peptides not only decrease the upper and lower values of blood pressure, but they also prevent their sharp increase.

d) Lactic acid fermentation on non-dairy substrates

In the recent 20 years lots of experiments have been made worldwide, in Japan especially, for cultivation of lactic acid microorganisms, *Lactobacillus bulgaricus* and *Streptococus thermophilusc* in particular, on non-dairy substrates: soy-bean products, peanut milk, wheat germs, etc. In the early 90s Bulgarian BAS scientists together with the ELBY Company developed a patented technology for fermented grain foodstuffs based on oats and Bulgarian yogurt symbiotic starter.
Recently the UFT – Plovdiv has developed a technology for obtaining a wholesome oats kind of drink, fermented together with other lactic acids microorganisms.

e) Dairy products fermented with other strains

The world market is supplied with a series of dairy foodstuffs fermented with other strains, bifid bacteria for instance. Similar products are currently offered on the Bulgarian market as well (by the world-known Danone Company) and they have great potential due to their wholesome effect on man.

2. Grains, bread and sugar

Bread yeasts are one of the most significant biotechnology products in our country – at present a great quantity of them is imported, part of them is produced in the Plant for bread yeast in Rousse. There is doubtless need of modernizing the manufacture or building and introducing new equipment. In the bread production process a number of food additives are used containing enzymes such as α-amylase, xylanase, etc. These enzymes were produced in the Enzyme Plant – Biodegrade and part of them was exported to the EU countries. There is reason for claiming that further development of enzyme and microbial technologies for the bread producing industries is necessary.

The grain drink boza (millet-ale) is a fermented product with long traditions in the region, however the identification of the microorganisms involved in the process was performed in the last 3 or 4 years by Bulgarian and German scientists. It is worth noting that at present there is no such product supplied on the market, for the wheat gruel that is currently sold on the market under that name is synthetically sweetened, preserved and is not obtained through fermentation. Scientific and technology research is

definitely necessary in order to obtain a fermentation product with its typical features as nutrition and its bioactive substances, and in the same time to meet the EU hygienic food requirements.

3. Beverage industry

The beverage industry has a long history and tradition in Bulgaria and holds a significant share in the country's GDP and is closely related with the biotechnology development. In the beverage industry (wine, beer and spirits) only cultivated yeasts technologies are used. There is apparent increase use of enzyme препарати that improve the production yield, clearness and stability of the drinks. Before 1989 primarily enzyme preparations produced in Bulgaria were used in the various branches of the food and beverage industries. After 1989 many foreign companies entered the Bulgarian market (producers and commercial representatives). The enzyme preparations range supplied by them was much wider compared to the ones locally produced, and that combined with the lower prices made their products much more competitive than ours.

In the brewery industry in Bulgaria a range of enzyme preparations is widely used for the following applications:

- Intensification of production processes by applying various enzyme technologies;
- Better use of the input raw materials;
- Increase of the nutritive and consumer value of the final produce;
- Expansion and diversification of a wider range of products.

Special attention should be paid to the vinegar production sector – a traditional spice obtained through biotechnology methods. In the last 3 or 4 years there has been a tendency to enrich vinegar with a number of food micro-additives and flavor plant extracts. On the other hand the world market demands high quality wine and fruit vinegar.

4. Special foodstuffs and additives

The better the nutrition culture and the awareness which food is healthful, the wider combinations of balanced and enriched foodstuffs would be created for various consumers with various needs. The development of this sector would gain such growth that would be comparable to the replacement of natural rubber with synthetic. In the last 5-10 years the sales of such foodstuffs have demonstrated a yearly growth of 15% and that trend tends to persist.

5. Canning industry

Enzyme technologies are applied in juice production and filtration and enzyme preparations for that purpose were recently produced in Bulgaria. A very perspective enzyme technology application is the decrease of undesired substances in the raw materials input. The elimination of oligosaccharide of raffinose type that hold 5-6% of the total weight of legumes seeds is a perspective research area. This kind of saccharides cannot be digested by human beings and non-ruminant livestock, they pass through the digestive system not being decomposed and going into the colon they stimulate the bacteria growth causing flatus. A technology for decomposition of this oligosaccharide was developed in Bulgaria using seed germination process during which the α-galactosidase present in the seeds hydrolyzes the oligosaccharides down to the digestible sugars galactose and saccharose The germs produced may further serve as source for the production of proteins concentrates, emulsions, pastes, etc., thus the price of the final products would be corresponding to the low incomes of part of the population. The presence of vegetables with delicious taste makes it possible to produce a wide range of canned vegetables

enriched with digestible proteins and sugars at low price level. The Danish NOVO ENZYMES Company is currently developing a method for the production of α-galactosidase of microbial origin, which would significantly facilitate the technology.

6. Waste product treatment

The waste products from the food, wine and tobacco industries are a serious problem to environment protection and their treatment in most cases is targeted towards their elimination and to smaller degree towards an economic efficiency. Most waste products come from slaughterhouses, whey, beer yeasts, grape seeds after wine production, etc. There are researches for their utilization currently carried out in Bulgaria, that have to be revised in accordance with the EU requirements, regarding waste products of animal origin in particular.
The waste yeasts from the brewery industry are a good example of a product with high biological value, which is applied in the production of protein and vitamin products used in food and pharmaceutical industries, in biotechnology and agriculture. Its utilization allows the decrease of the biological substance in waste water and thus contributes to environmental protection. The protein hydrolysates, yeast extract, etc., can be used both independently and as prophylaxis medical additives or for the production of functional, diet or curative food designed for particular groups of customers. The above mentioned products are produced in Bulgaria, in small quantities though, and the yeast autholysate is used in the Biovet Company – Peshtera.

B. AGRICULTURE

1. Aquacultures

Aquacultures are a very interesting area of biotechnology methods application, most popular of them is fish-breeding. In some European countries there are on-going scientific researches on the cultivation of algae and the utilization of algae from natural sources. Bulgaria is the only European country that has a functioning semi-industrial installation for cultivation of *Chlorella* and *Scenedesmus* green algae. Their annual production has reached up to 7 tons dried biomass intended for export or for processing and obtaining of protein hydrolisates for regenerative foods and pharmaceutical products.

2. Biotechnology in livestock breeding

a) Biotechnologies and DNA-analyses

- Gene mapping
  Gene mapping is rapidly growing molecular technique for determination of most economically important livestock species: cattle sheep, pigs, poultry, fish, silkworm, etc. As for instance gene mapping technology elaborated for cattle breeding purposes provides possibility for determination of some polimorphic loci at DNA level such: kappa-casein locus, somatotropin, inter-loci association of some molecular genetic systems, genetic differentiation in cattle as related to influences of biotic and abiotic factors of ecological stress.
- Marker Assisted Selection (MAS)
  Methodical principles of marker assisted selection (MAS) apply molecular genetic techniques for development of better conditions for livestock improvement. MAS is biotechnological addition to current selection programmes. A new selection tool – marker is included in the programmes through these selection methods. Selection is managed by a complex index, involving the effect of polygenes and the effect of markers, related to sire and dam. MAS is based on the linkage between the markers and the loci determining the

expression of quantitative traits. Analyses employs the methods of regression analysis, segregation analysis, maximum likelihood and Gibbs sampling. BLUP method can be used for breeding value estimation, which at the same time eliminates the effects of the environment and by which the breeding value is estimated on the basis of polygenes and breeding values of the investigated loci, being robust enough. MAS can be applied especially in MOET (Multiple Ovulation and Embryo Transfer) selection schemes, linked up with extensively bred reference herds. It brigs up an increase of the genetic gain up to 2 – 30 %.

- Optimisation of response using molecular data
- Using DNA-technology for identification and testing animal infections and genetic diseases by different pathogenic agents
- DNA-technology and evaluation of philogenic interrelations between domestic and wild mammalian species

b) Biotechnologies and reproduction efficiency

- Improvement of methods for cryoconservation of male gametes, species specifically oriented. The media for crioconservation (diluter) of male gametes is improved. Thus it could be utilised in two directions:
  - intensification of selection process (wider use of best genetic material).
  - Conservation of genetic resources.
    Regional GenBank for long-term conservation of gametes. This is of big importance for small local breeds as well for testing of the genetic changes as result of selection activity.
- Intensification of reproduction ability in females. This is related to the increase of the ova number per animal (Multiple Ovulation) or to In Vitro techniques by splitting and In Vitro techniques for maturation (IVM). From breeding point of view all these approaches are oriented towards obtaining more progenies of genetically superior females. Some of these technologies are major subject of private business of small to average enterprises. These techniques gained large interest for rapid improvement in livestock among producers of the livestock society.
- Sex determination. Early detection of sex chromosome and some related characteristic of gametes, provides possibility for sex splitting – to regulate the sex ratio in a generation of bred animals.

c) Biotechnology in disease resistance

Genetics of disease resistance now becomes one of the major focus areas within animal genetics. There is a need to assess the role of genetics to assist in the control of disease problems and both "classical" and molecular technologies are available for such a prospect. The main questions here are; which diseases to address, how to collect the required data, how to exploit the genetic knowledge and how to evaluate the consequences of selection. Finally the disease problems must consider both animal genetics and disease epidemiology, reduction in disease incidence, severity and transmission

3. Biotechnologies in plant growing

a) Plant Protection

For more than 40 years a considerable number of Bulgarian researchers have worked on the biological method for disabling the pests in agriculture. Technologies for producing biomatter against pests in agriculture have been developed and further improved in Bulgaria. There is wide application of biotechnologies in the emerging bio-plants and bio-laboratories. They are not currently functioning

yet. The Gene-bank of isolated microorganisms and microorganisms originating from the soil micro-flora was destroyed. At the same time private laboratories were set up such as Agrobioconsult – Sofia.

In the field of plant protection there have been research and technology developments in two main directions:

- Macroorganisms – parasites and predators
- Microorganisms – viruses, fungi and bacteria

1. Macroorganisms

1.1. Parasites

The following families have significant application:

- **Parasites of the Trichogrammatidae family**

  Leading research organization in this field is the Institute of Plant Protection in Kostinbrod

  Bulgaria has imported from Russia a biotechnology for trichogramme production. This biotechnology has been further developed and widely applied in four bioplants: Stara Zagora, Veliko Tarnovo, Pleven and Plovdiv and in the biolaboratories in Lovech, Razgrad, Vratsa, Varna and Montana. In 1990 trichogrammed crops reached 2300 thousand decares (maize, cabbage, beetroot, etc.). There has been biological treatment against corn stem borer.

- **Parasite insects of the Aphelinidae family**

  RTD is carried out in the Institute of Plant Protection in Kostinbrod, the Institute of Canning Industry – Plovdiv. Wide application is witnessed on over 6000 decares of steel and glass greenhouses for production of tomatoes and cucumbers primarily. This biotechnology method has been applied against the most devastating parasite insect of this family.

1.2. Predator species

- **The Phytoseiidae family**

Perfected was a technology for production and vast application in greenhouses against pests of the Tetranychus species, adversaries on vegetable cultures.

- **The Chrysopidae family**

  Developed was a methodology for season colonization and produced biomass in laboratory condictions. There has been successful treatment against plant louse in the tobacco crops.

2. Microorganisms

2.1. bacteria

- **Bacillus thuringiensis Berliner**

  Production of biopreparations is done in the Plant in Peshtera. There has been wide application in the forests against leaf-eating caterpillar.

2.2. Enthome pathogenic fungi

3. The Trichoderma genus

In Bulgaria a production technology has been developed in the Plants in Petrich and Plovdiv. The biomass produced has been used on 3000 decares of greenhouses as part of the micro-biological method for protection against weeds.

The renovation of bioproduction for plant protection would be of great benefit to Bulgaria, especially if it is performed in compliance with the EU requirements for ecological agricultural produce.

b) Plant Biotechnology

In the field of **Plant Biotechnology and Plant Genetic Engineering** the leading institution is the Institute of Genetic Engineering, Kostinbrod that in 1999 was selected as Centre of Excellence in Plant Biotechnology (EXCELLENT PLANT BIOTECH ICA1-1999-70003). The main thematic directions in the Institute are:

- Plant genetic resources,

- Phytopathology, Ecophysiology,
- Biochemistry, Cytogenetics,
- Molecular genetics, Functional genetics,
- Bioinformatics
- Micropropagation and tissue culture,
- Breeding and introduction, Breeding and technology of carnation,
- Agrochemistry,
- Potatoes breeding and production and Flax breeding and seed production.

The institute initiated the development of the National safety regulations for the utilization of genetically modified plants (GMP). The draft of the Living Modified Organisms Act has been prepared with the active participation of ABI and is now being discussed in the government and the Parliament. For its activities and initiatives in the area of biosafety and biodiversity ABI has been selected as sub-regional center for Eastern Europe in the field of regulations and a control of GMO.

ABI registered a number of practical achievements in the field of plant biotechnology. Resistance to diseases and stress factors has been genetically engineered in alfalfa, tomato, tobacco, grape, barley, carnation, apple and other crop species. Some of these are expected to be approved as the first transgenic cultivars in Eastern Europe by the year 2002. Specific molecular markers have been identified for originally created CMS in sunflower, tomato and tobacco.
ABI competence and experience in the field of plant sciences in general has provided the grounds to select the institute to organize a new **accredited laboratory**. The laboratory is established to assess and propagate certified phytosanitary pure and genetically authentic plant material of economically important plant species (grape, potato, strawberry, raspberry, rose etc.), to trace genetically modified product by DNA analysis and to assess forage quality by NIRS analysis.
Recently ABI started the initiative to organize together with Sofia State University "Kl. Ohridski", Faculty of Biology, NATIONAL AGROBIOTECH PARK. The idea is to found a modern research park in Sofia, Bulgaria which will be concentrated on plant biotechnology, biosafety and genomics.
AgroBioTech Park will be a pilot project for Bulgaria and South-East Europe.
AgroBioTech Park will be a connection which will realize the scientific achievement in agriculture, food industry and solving the problems of ecology and health care.
The establishment of AgroBioTech Park on the territory of the Biological Faculty will improve the education of students to meet the challenges and competition of the world science and business market.
The major goals of the initiative are as follows:
1. To support the development of an independent, competitive and efficient plant biotechnology capability in Bulgaria.
2. To utilize the Plant Biotechnology expertise and know-how currently available and to foster further development in this area of science.
3. To support the transfer of technology for the improvement of agriculture and the solution of local and regional agricultural, ecological and health care problems.
4. To provide an incubator for the establishment of new plant biotechnology and genomics based companies. The AgroBioTech Park in Sofia could be an incubator for the establishment of new joint venture companies between international state organizations and private companies.
5. To provide a center of excellence in Biosafety and Biodiversity and a regional training center for public officials and the private sector in this areas.
6. To provide an environment that will attract research investments by national, regional and multinational Plant Biotechnology companies with a focus on Bulgarian and South-Eastern European agricultural needs.

IX. Conclusions

One of the key priorities of the Government Programme is the adoption of a national integrated strategy for the sustainable development of agriculture compliant with Bulgaria's specific and unique physical features and with the EU Common Agricultural Policy. New production methods and a new investment policy are to guarantee the food safety in the agricultural products segment, make the agricultural products more competitive.

In line with the priorities the government has launched the Genomics Programme, partially funded by the EU (Managed by the Ministry of Science and Education) targeted towards the development and expansion of activities in the area of biotechnology. The participation of the country in the European Frame Programmes is the legislative base for future integration negotiations on Chapter 17 Science and Scientific Research.

There is a crucial need for enhancing and facilitating the development of national capacities to assess and manage the current and future development of biotechnology, need for development of expert human resources in biotechnology, strong need for increased capacity building programme in this area. The implementation of the foresight exercise should provide useful additional experience and expertise in Bulgaria. The official authorities in the country encourage the development and application of modern biotechnology in the context of general market economy in the country. Local authorities and the general public are also supportive owing to the importance of biotechnology to the economy of the country.

The present survey clearly showed that Bulgarian biotechnology is now in a stage of slow recovery. This process proceeds most successfully in the field of fermentation biotechnology dedicated to manufacturing of antibiotics and other pharmaceuticals, which is one of the first privatised industries in the country. The other areas of biotechnology are still lagging behind because of the lack of booster funds. The next branch, which is expected to be recovered soon after privatisation, is the mineral biotechnology. All technologies applied so far in this field (bacterial leaching of rare and expensive elements) have been developed at the University of Mining and Geology and are well known abroad. Some of them have been bought and successfully applied in other countries (like Brazil). A promising field of biotechnology seems to be the genetic engineering of biologically active substances for medical use. Taking into consideration that these substances (most of which are hormones, immunomodulants, growth factors and regulators, etc.) are endowed with high biological activity, their manufacturing requires modern pilot plants rather than real big plants. They are profitable since they themselves, as well as their final (ready for use) forms are still quite a bit expensive. The world market for this category of products is estimated to be several hundreds of billions of US dollars for the next few years. Genetic engineering, however, requires educated and highly qualified people. As already mentioned, such specialists are presently available in the country and are continuously preparing at the Bulgarian universities and research institutes. A key consideration in favour of developing such a high biotechnology in Bulgaria is the low price of the qualified labour and the cheap clinical trials in the country. According to the forecasts this situation will remain almost unchanged during the next few years.

On the basis the Government Programme key priorities and the background information on the status of technology and innovation development in the area of biotechnology in Bulgaria the following sub-areas were identified, which the foresight pilot initiative in Bulgaria should be focused on: the food and beverage industry and agriculture, i.e. livestock breeding and plant growing.