Symbiosis, 2 (1986)
Balaban Publishers, Philadelphia/Rehovot

Impulse to European Cooperative Research in the Field of Agricultural Microorganisms; Interactive Programme for Interactive Microbes

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Abstract

The Biomolecular Engineering Programme (BEP) for the European Communities has been the focus for intense activity in genetic engineering of species relevant to agriculture and agro-food production during the last four years (1982–1986). For the years ahead, the European Commission will maintain its support for cooperative research in the same fields through the implementation of the new Biotechnology Action Programme (BAP : 1985–1989). The application of genetic engineering to symbiotic or pathogenic microorganisms therefore remains a major focal point, because of the economic challenges it brings to agriculture, and also for its potential capacity to unravel many aspects of plant molecular biology.

Keywords: Europe, Biotechnology, transnational research, lignocellulose, plant-vector, nitrogen fixation

1. Introduction: Evaluation of an ending programme

The European Commission launched in 1982 a research and training programme in the field of Biomolecular Engineering, known as "BEP" in the scientific world. It was really the first Community initiative as far as Biotechnology was concerned, sill modest in its appropriations and therefore concentrated on objectives considered most urgent and important for Europe. BEP was consistently focused on the development of methods,

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0334-5114/86/$03.00

based either on genetic engineering or on enzyme engineering, which would contribute, through the modification of organisms important for agriculture or agro-food production, and through the improvement of fermentation or transformation processes, to the long term establishment of new foundations for the Common Agricultural Policy.

The chosen method of implementation was to create, through pooling materials, ideas and skills dispersed throughout Europe, small Community research nuclei concentrating on specific scientific topics. Because it could virtually abolish geographical distances and state boundaries, BEP could impose on all participating laboratories the common rules providing organic bonds to secure the assembly of such research nuclei. "Common rules" may relate to periodical participation in joint working parties, the dissemination of unpublished information, exchanges of material or staff whenever appropriate, the evaluation and publication of results in common as much as possible. No doubt this idealistic view was implemented in the framework of BEP with a variable degree of success, and there are certainly research nuclei which proved to be more convincing than others. One area of the programme where transnational collaborative research was most active and efficient was that concerned with symbiotic or pathogenic microorganisms. Twenty-four laboratories out of a total of 103 concentrated on these topics. They can be subdivided into 3 major research nuclei:

- Four laboratories cloning cellulase and pectinase activities from *Erwinia* spp.;
- Nine laboratories developing vectors for plant genes from pathogenic microorganisms (*Agrobacterium* spp., Cauliflower mosaic virus, satellite tobacco necrosis virus);
- Eleven laboratories unravelling molecular bases of growth-promoting symbioses (*Rhizobium* spp., mycorrhizae, siderophore-carrying *Pseudomonas* spp.).

These groups were able to integrate their research work in a rather exemplary manner, as testified by the scientific achievements reported below.

2. Interactive Microbes

All these microorganisms, irrespective of their impact on agricultural productivity (which is substantial), have a very basic quality for the molecular biologist: they have evolved natural systems of communication with plants, based on the very principles of genetic engineering or of enzyme engineering which the biotechnologist is trying to exploit in his artificial environment.

The way in which these microorganisms operate their associative mechanisms can teach us a lot about genetic domestication, and the following details illustrate what our groups of contractors could learn in this respect:

Pathogenic microorganisms

Ligno-cellulose degradation

Laboratories in Brussels, Paris, Lyon and Marseille distributed the tasks between themselves to unravel the system by which *Erwinia*, causative agent of the soft rot disease, produces and secretes extracellular enzymes responsible for early stages of pathogenicity. They developed genetic techniques, including host-vector systems, for *Erwinia* species which are now amenable to genetic analysis. They cloned and started to characterize 5 genes encoding pectate-lyase isoenzymes, one gene encoding a pectine-methylesterase (all pectinolytic activities) and identified at least one gene coding for a cellulase. This is a real research nucleus, in the sense that it brings together complementary skills (plant pathology, biochemistry, enzymology, molecular genetics) from Institutes located in different countries, and developed into a platform of unique know-how for a range of possible exploitations in the future: production of anti-tumoral enzymes, construction of secretion vectors for the production of engineered polypeptides, exploitation of *Erwinia* pectinase/cellulase genes to increase substrate utilization of industrial microbes, identification of mechanisms underlying plant phytopathogenicity.

Plant-vector development

The bacteria *Agrobacterium tumefaciens*, responsible for the crown-gall disease, and *Agrobacterium rhizogenes*, responsible for the hairy-root disease, are now well known as natural microinjectors of foreign DNA into the genome of infected plants. Progress in exploiting these natural transfer systems for the genetic manipulation of plants, or for the development of physiological and genetic investigations in transformed organisms, has been more rapid indeed than any increase in the knowledge of the underlying infection and transformation mechanisms. Work carried out in Rome, Orsay, Versailles, Ghent, Leiden and Harpenden contributed to broaden the spectrum of economically important species now amenable to transformation by *Agrobacterium*, including potato, tomato, carrot, soybean, alfalfa, *Lotus*, sugar beet, oilseed rape, asparagus, etc... The feasibility of the method mainly depends upon the use of disarmed vectors, which are plasmids derived from the original Ti or Ri plasmid from which oncogenes are deleted and substituted, in particular, by selectable marker genes an genes of any interest for the final product. New generations of vectors are being developed, simplified in the sense that they harbour ever fewer original sequences from the Ti-plasmid (the laboratory in Leiden showed the possibility of removing virulence genes acting in TRANS in a binary vector system, and the group of Ghent could restrict to a 24 bp right border the sequence essential for integration), but more sophisticated also by the fact that plant inducible promotors are becoming available to substitute the strong constitutive promotor normally carried by the T-DNA.

In this sector also, transnational cooperation proved to be of key importance. The benefit can be seen at 2 levels at least:

- Expert laboratories in Ti/Ri vector developments (Ghent, Cologne, Leiden, Orsay, Rome,) are generally distinct from expert laboratories in plant gene isolation and characterization (Harpenden, Aarhus, Amsterdam,), and collaboration between them is obviously directly symbiotic. This can be exemplified by referring to one of the successful transnational experiments in which BEP played a significant role: a selectable marker gene with a leghemoglobin promotor from Soybean (Aarhus), transferred to *Lotus* with the Ri plasmid system (Cologne), could be specifically expressed in the nodules of the transformants in presence of *Rhizobium*, repressed in all other organs of the heterologous host.
- The characterisation of oncogenes, carried either by Ti or Ri T-DNA, and of their effects on plant cell responses, is another critical area at the forefront of plant science, where laboratories working on various strains of *A. tumefaciens* and *A. rhizogenes*, different host species and different organs of the hosts, need to carry out comparative assessments of their observations and to exchange sequence data or molecular probes.

This multidisciplinary sector, also with a range of multiple applications, allows for variable collaborative configurations, from targeted tandem associations to more "horizontal" brain stormings such as organized with J. Tempé in Orsay in September 1984 (14 BEP contractors convened).

3. Symbiotic microorganisms

Nitrogen-fixing symbiosis between Rhizobium and legumes

The question is whether beneficial soil microorganisms can be engineered to the extent that they would entirely serve the needs of the plant for a stabilized or even an increased productivity. Is it possible, in other words, to convert

the microsymbionts into an extra-organ of the cultivated macrosymbiont, carrying out specialized functions such as the displacement of pathogenic populations in the soil or the improvement of mineral/water nutrition. A specialized organ is maintained through organic links, which, in the case of symbiotic microorganisms, will have to be sought at the level of genetic and metabolic cooperation mechanisms. These genes and metabolites do represent the targets for contractual research under BEP. Nine laboratories made convergent efforts in investigating the genetic controls of nodulation and of nitrogen fixation in *Rhizobium* species associated with legumes important for European agriculture (alfalfa, pea, faba beans). The number of genes isolated, and on the way of being characterized, is becoming impressive:

- 8 nodulation genes of *Rhizobium leguminosarum*; sequence data and product isolation already give clues to their possible functions (Norwich);
- various Fix and Nif genes in *Rhizobium meliloti* and *Rhizobium leguminosarum*, with preliminary evidence that genes controlling nitrogen fixation, unlike nodulation genes, might be far more dispersed in the genome (Fix genes located 200 kb remote from the main Fix/Nod region of the symbiotic plasmid shown by a contractant of Toulouse; presence of a non-symbiotic plasmid contributing to the modulation of the nitrogen fixation efficiency demonstrated by a contractant of Erlangen);
- soluble and non soluble proteins shown to be specific to the root hair of legumes, a highly significant result when one considers that the root hairs are the plant cells first recognized by the bacteria (Marburg);
- organization and coordinated expression of soybean leghemoglobin genes largely elucidated (Aarhus);
- 14 nodulin genes in soybean and other nodulin genes in faba beans (Aarhus and Bielefeld), etc.

A very particular feature of the transnational cooperation in this third sector is the exceptionally intensive exchange of molecular probes for the series of Nod, Nif and nodulin genes, speeding up considerably the scientific progress in these laboratories working with different microbial strains and different plant species. Although the construction of improved symbiotic strains remains the ultimate goal, this Community nucleus of research is presently contributing significantly to the development of a model for plant-bacteria recognition, which will be central to any engineering approach in the rhizosphere.

Other beneficial interactions with soil microorganisms

Exploratory work has been supported in the framework of BEP to define the feasibility of other research avenues with microorganisms not so well described from the point of view of molecular genetics.

A laboratory in Utrecht demonstrated the growth promoting effect of siderophore-producing *Pseudomonas* on potato, as well as the direct causal relationship between siderophore production and the effect observed. Although the role of siderophores with regard to competition with pathogenic microorganisms or to plant iron nutrition is not clear yet, there is scope for a genetic engineering approach in this system.

Ectomycorrhizal fungi (*Morchella conica* and others) were studied in Bochum, and might offer prospects for extrachromosomal genetics since linear plasmids could be isolated.

Lignocellulose degradation, plant vector development, symbiotic associations in the soil: these were prominent scientific issues in the Community programme, giving rise to the constitution of transnational research nuclei where measurable results can be ascribed, at least partly, to the integration of research efforts across state boundaries.

In no way would this conclusion imply that the major achievements listed above should be ascribed to the sole merit of BEP. Considering the low amount of funding provided by the programme, it is only reasonable to assume that the contribution from the Community budget is a limited part of the means allocated to research projects as a whole. However, BEP can claim the catalytic effect it provided, by creating a climate altogether favourable to innumerable exchanges, bringing about a definite acceleration in the progress of strategic research.

4. Interactive Programmes

After this sketchy review of research activities on agricultural microorganisms boosted through the implementation of BEP, it should be shown how the Community programme has been attempting to provide the range of interactions which would suit a multidisciplinary field with predictable, as well as unpredictable, fall out. BEP had undoubtedly too small a structure to cope with the challenge for innovation met by agro-industrial sectors.

A new programme was launched in 1985 to amplify the effort initiated with BEP, the Biotechnology Action Programme (BAP: 1985 — 1989), embracing

training, research, development and concertation actions. This new initiative has been tailored to afford interactions and complementary measures at different levels: a simplified picture could be drawn by distinguishing two interactive levels of horizontal and vertical nature, as well as a more global and integrated approach.

Horizontal level

BAP will accommodate quite a large number of different research sectors, involving the whole range of modern techniques (bio-informatics, enzyme and protein engineering, genetic engineering, technology of cultured cells), and addressing a wide variety of biological systems relevant to all sorts of agricultural and industrial processes. This is a significant increase in multidisciplinarity compared to BEP, offering exceptional opportunities for horizontal technology transfers. The services of the Commission actively contribute to removing barriers between academic disciplines and organismal researches, by organizing on appropriate occasions multisectoral contractors meetings of reasonably small dimensions (± 100 participants). This exercise was already attempted in the framework of BEP on two different issues:

- common applied interests (Biomolecular engineering for agriculture, Louvain-la-Neuve, November 1983).
- common methodological obstacles (Plasmid instability, Heraklio, October 1985)

Vertical level

BAP is the occasion for the Commission to put an increased emphasis on the vital need for intense technology transfers between basic science and industrial research in Europe. An outcome of BEP has been the emergence of a multinational platform of basic methodological researches, which will be further strengthened in the framework of BAP.

It is essential to facilitate access by industries to collaborative precompetitive research. Industrial scientists can easily grasp any scientific result coming to their knowledge, when the source of know-how is localized precisely. When dealing with collaborative research however, the involvement of industries requires a multilateral communication process in which the Community programme can play a very decisive role.

BAP is sufficiently qualified as a place of continuous dialogues and exchanges between those who invent technologies, those who transform technologies, those who exploit technologies eventually. The implementation methods of the Community programme foresee simple and flexible mechanisms for this dialogue to take place, including consultative meetings, joint scientific meetings, sponsoring of academic research, access to scientific information, and contractual arrangements allowing the involvement of industry in pre-competitive research work or its contribution to funding the projects.

Global approach

Interactions are also required between research and development (R&D), in the strict sense, and a number of environmental factors controlling scientific information and its translation into applications. BAP has been integrated in a framework of actions complementing R&D, whereby the sustained development of biotechnologies can contribute to the socio-economic objectives of the Community. Certain actions are included as elements of BAP, others are treated separately by competent Directorates-General of the Commission.

Subprogrammes complementing basic biotechnology R&D in the framework of BAP

- Contextual measures for biotechnology R&D is a subprogramme of BAP taking care of the infrastructures for biotechnological research in Europe. It focuses on the development of software for modelling predictive capabilities for biological systems and processes, on the upgrading and integration of biological data banks, on collections of biotic materials. Among the collections supported to date, one can quote:
  - human genetic mutants
  - human lymphoblastoid cell lines
  - lactic acid bacteria
  - plasmid-bearing bacterial strains
  - fungal strains
- Concertation of biotechnology policies in the Member States and at Community level: this is another action, also part of BAP, which seeks to improve strategic awareness and to promote greater coherence between all areas of policy impinging on biotechnology.

Separate actions covered by other Directorates-General

- New regimes on agricultural outputs for industrial uses (DG III, DG IV)
- Regulations affecting biotechnology (DG III, DG V, DG VI, DG XI)

- Intellectual property rights in biotechnology (DG III, DG IV, DG VI, DG XIII)
- Demonstration projects (deferred provisionally)

A Biotechnology Steering Committee, chaired by the Director General for Science, Research and Development (DG XII), provides the necessary harmonization between these actions, in which various sectoral policies converge. This is the ultimate level where "interaction" lights up as the magic keyword.

5. Conclusion — Good wishes to a starting programme!

In response to the call for research proposals issued in the framework of BAP in February 1985, the services of the Commission received more than 1500 projects by the deadline of 31st March 1986, with a proportion of 80% which appeared combined right from the beginning into transnational proposals. The expansion of BEP into BAP was followed by such a mobilization of research capabilities in Europe, that the new programme will be faced with the acute problem of being still more underfunded than it was in the past (1 proposal accepted out of 3 in BEP, probably 1 out of 7 in BAP). The balance of priority research efforts will have to be adjusted accordingly, by sticking to areas of strategic significance. One of these areas will certainly be that of symbiotic and pathogenic microorganisms, not only because of the economic challenges it will bring to agriculture, but also for its potential capacity to unravel many aspects of plant molecular biology. In discovering the ways in which these microorganisms exploit existing plant mechanisms, one may find clues to the ways in which plants regulate themselves. The interface of plant-microbe interactions is the place of an exchange of active molecules which could determine a range of cellular responses in the host-plant, pertinent to cell wall synthesis, on-set of cell divisions, morphogenesis.

The subject is therefore strategic both from an agricultural point of view (applications) and from a basic point of view (knowledge).

S.R. Long stressed this fact in very convincing words: "The molecular and genetic study of symbiosis may have significance beyond the particular systems in question. In molecular genetics, it has often been the case that an understanding of cell function is advanced by research on pathogens. This is apparent in the contributions of phage research to our understanding of bacterial molecular biology, or of research on toxin-producing microbes to our knowledge of membrane function. This may prove to be the case with a symbiont such as *Rhizobium*".

Provided they do not loosen their collective bonds which proved so successful, the existing transnational research nuclei can build up into European centres of excellence which both academic and industrial communities would welcome.