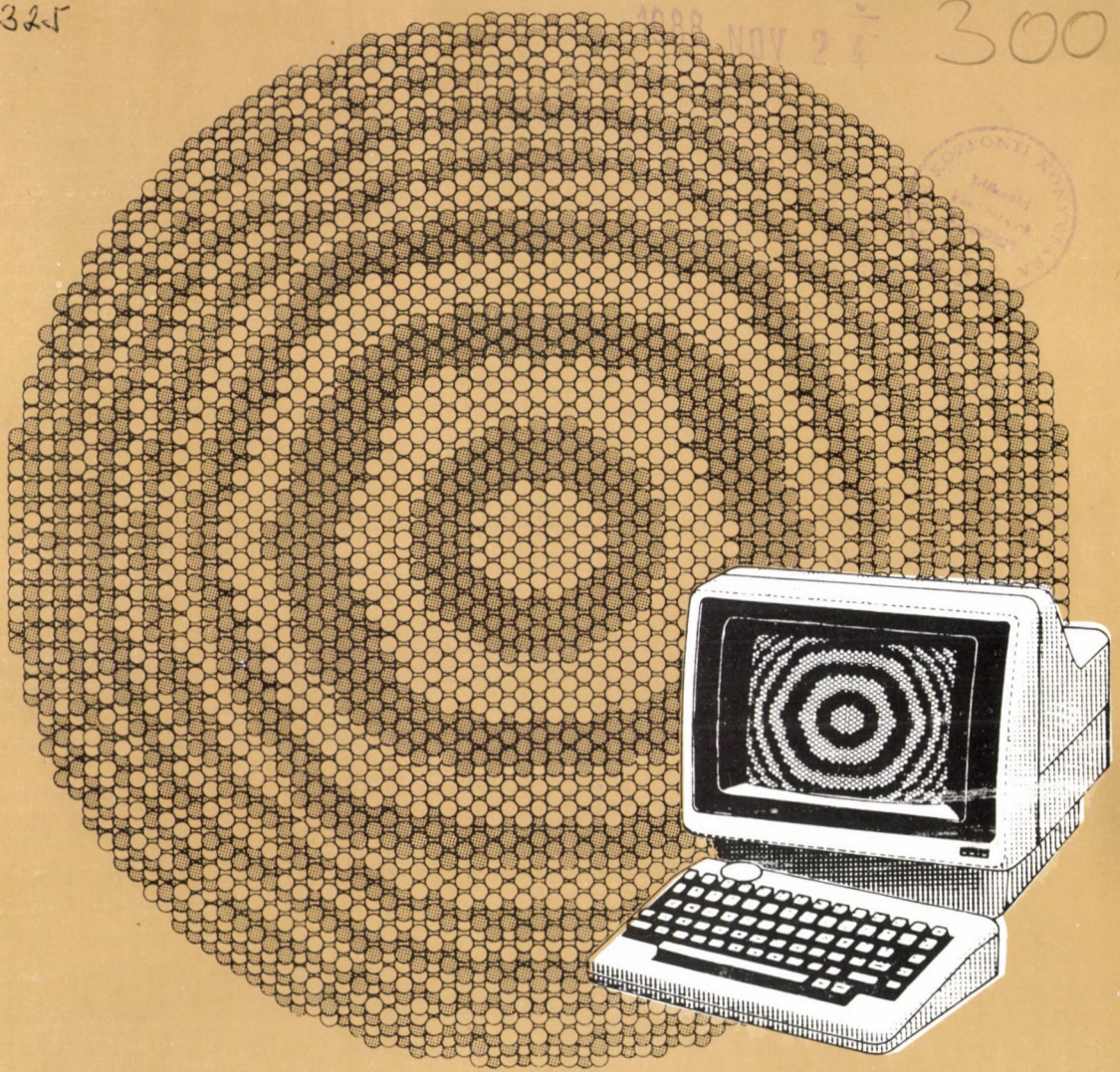

biotech-info

Special issue 1987. HU ISSN 0237–0115

OMIKK AGROINFORM

BIOTECH-INFO

1987.
Special issue for foreigners

The publication contains abstracts and annotations of selected works published by Hungarian authors in the technical literature on biotechnology in Hungary.

EDITED BY THE NATIONAL TECHNICAL INFORMATION CENTRE AND LIBRARY (OMIKK) – AGROINFORM – NATIONAL COMMITTEE FOR TECHNICAL DEVELOPMENT (OMFB)

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Készült
az OMIKK házi nyomdájában
(Budapest I., Gyorskocsi u. 5– 7.)
Felelős vezető: Tóth Károly

300

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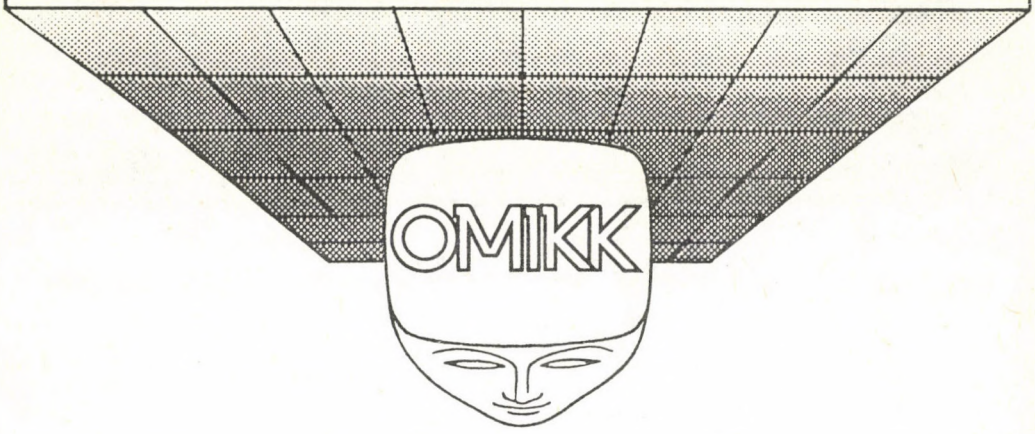

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3. **Biotechnology Today:** these publications are studies, review of general interest on biotechnology as well as on materials of different seminars, conferences and symposiums. There are about 6 issues in a year. Hungarian title: Napjaink Biotechnológiája.

GENETIC ENGINEERING

FLOW CYTOMETRIC DETERMINATION OF THE SPERM CELL NUMBER IN DILUTED BULL SEMEN SAMPLES BY DNA STAINING METHOD

/T.Takács, J.Szöllősi, M.Balázs, R. Gáspár, L.Mátyus, G.Szabó, L.Trón, I.Resli and S.Damjanovich/

Acta Biochimica et Biophysica Academiae Scientiarium Hungaricae, 1987. Vol.22. No.1. p. 45-57.

Flow cytometric determination of the number of bull sperm cells showed that the number of spermatozoa measured by light scattering may considerably differ from the actual number of sperm cells in the samples, depending on the proportion of contaminating particles, similar in size to sperm cells. No accurate information can be obtained from the sum of live and dead cells distinguished by means of double fluorescence staining, since a part of the sperm cell population is in a transitory state i.e. between the viable and dead states, so it cannot be stained by either dye.

The number of spermatozoa in the sample can be determined very accurately if the sample is treated first with Nonidet-P-40 detergent then stained with propidium iodide. With this procedure the DNA content of each cell nucleus can be labeled and, through detecting the fluorescence signals, the actual sperm cell count of the sample can be determined with the accuracy of 95-98 %.

ISOLATION OF HIGH MOLECULAR WEIGHT PLANT NUCLEAR DNA SUITABLE FOR USE IN RECOMBINANT DNA TECHNOLOGY

/T.Kiss, F.Solymosy/

Acta Biochimica et Biophysica Academiae Scientiarium Hungaricae, 1987. Vol.22. No.1. p. 1-5.

Nuclei isolated from leaf cells of broad bean /Vicia faba L/ by a newly developed method based on the use of citric acid in the isolation medium and floatation on a Percoll cushion yielded high molecular weight plant nuclear DNA which was suitable for /i/ analysis by restriction endonucleases, /ii/ molecular cloning and /iii/ genomic blot hybridization. Starting from nuclear preparations obtained by this method, U2 small nuclear RNA-specific DNA sequences were de-

tected in Vicia faba L. This is the first report on the demonstration of small nuclear RNA-specific DNA sequences in plant material.

EFFECT OF THE pKM101 PLASMID ON THE REPAIR OF SINGLE-STRAND BREAKS IN DNA INDUCED BY IONIZING IRRADIATION IN ESCHERICHIA COLI.

/I.Francia, Zs.Hernádi, M.Szabolcs, F.Hernádi/

Acta Biochimica et Biophysica Academiae Scientiarium Hungaricae, 1987. Vol.22. No.1. p. 85-97.

The effect of pKM101 plasmid on repair of singlestrand breaks in DNA induced by ^{60}Co-gamma irradiation in E. coli K12 AB1157 /wild type/ and in its recA$^-$ and gradient sedimentation method. For quantitative analysis of sedimentation profiles we calculated the $S_{1/2}$ values described by Veatch and Okada.

The $S_{1/2}$ values of unirradiated cells were 21.10, and after 200 Gray irradiation 11.35, due to the original incidence of single-strand breaks. The presence of pKM101 did not influence these values in either cases. This means that pK101 had no effect on the rise of single-strand breaks in DNA.

During a post-irradiation incubation period at 37°C for 60 min the $S_{1/2}$ value of the wild type strain increased from 11.35 to 19.22, that of the recB$^-$ from 11.50 to 15.23, while the $S_{1/2}$ value of the recA$^-$ mutant did not change owing to the lack of repair of single-strand breaks. pKM101 plasmid markedly increased the $S_{1/2}$ value in wild type strain and in recB$^-$ mutant, while it had no effect on $S_{1/2}$ in recA$^-$ cells, during this post-irradiation incubation period. Thus the effect of pKM101 on the repair of single-strand breaks in DNA proved to be dependent on recA^1 genotype.

Nalidixic acid at 100 μg/ml concentration inhibited the repair of single-strand breaks in both wild type and recB$^-$ mutant cells harbouring pKM101 plasmid.

CHARACTERIZATION OF THE L1NH REPEAT FAMILY OF NOVIKOFF HEPATOMA

/L.Tora, I.Financsek and J.Hidvégi/

Journal of Molecular Biology, 1987. No.197. p. 1-9.

Long interspersed repeated sequences of the Novikoff hepatoma rat tumour cell genome were cloned and studied. No basic differences were found when the genomic organization of the Novikoff hepatoma was compared with that of other mammalian L1 families. The nucleotide sequence of the central 4kb /1 kb=10^3 bases/ part of the Novikoff hepatoma LINE /L1NH/ appeared to be more highly conserved than the sequences found at the 5' and 3' ends. Moreover, the central 4 kb core fragments were not always associated with the same end sequences. Thus, the occurrence of the more-conserved and more-abundant central portion in L1NH suggest that: /1/ besides reverse transcription, other DNA- and/or RNA-mediated mechanisms might be involved in the dispersal of LINE families; and that /2/ L1 sequences can sometimes

consist of a compound unit made up of members of different L1 subunits and sequences with different genomic copy numbers.

INTERSPECIES HOMOLOGY OF NODULATION GENES IN RHIZOBIUM
/F.R.Quinones, Zs.Bánfalvi, P.Murphy and A.Kondorosi/
Plant Molecular Biology, 1987. Vol.8. p. 61-75.

The internal structural portion of genes nodC and nodD /representatives of the two transcription units coding for common nodulation function/ and of hsnB and hsnD /genes from the two transcription units determining host-specificity of nodulation/ have been cloned from Rhizobium meliloti into M13 vectors and used as probes against genomic DNAs from different Rhizobium strains and species. nodC and nodD were found in all species with one exception, indicating that they are common and widely spread genes, though the nodD gene hybridized only very weakly with slow-growing rhizobia. Interestingly, reiteration of nodD sequences was observed in almost all fast-growing strains /with the exception of R.leguminosarum/. hsnB and, more so, hsnD are present only in a few species tested, supporting their specific involvement in R. meliloti-Medicago sativa symbiosis. In several cases the hybridizing bands from total Rhizobium DNA were compared to those found in recombinant plasmids carrying functional nodulation regions, and these analyses supported the notion that the bands indicate the presence of functional genes.

GENES FOR THE CATABOLISM AND SYNTHESIS OF AN OPINE-LIKE COMPOUND IN RHIZOBIUM MELILOTI ARE CLOSELY LINKED AND ON THE SYM PLASMID
/P.J.Murphy, N.Heycke, Zs.Bánfalvi, M.E.Tate, F.de Bruijn, A.Kondorosi, J.Tempé and J.Schell/
Proceedings of the National Academy of Sciences, USA, 1987. Vol.84. Jan. p. 493-497.

In alfalfa nodules induced by Rhizobium meliloti strain L5-30 the compound L-3-O-methyl-scyllo-inosamine /3-O-MSI/ is synthesized. This compound is also catabolized specifically by this strain. Its biological properties are therefore similar to the Agrobacterium opines. To answer the question whether opine-like compounds /"Rhizopines"/ play a role in a plant symbiotic interaction, we isolated the genes for the catabolism of 3-O-MSI /moc genes/ and for the induction of its synthesis in the nodule /mos gene/s//. moc and mos genes were shown to be closely linked and located on the Sym plasmid of L5-30, suggesting that they have co-evolved and may be important in symbiosis. These genes have been cloned into a broad host-range vector that can be mobilized into other R. meliloti strains where they are expressed. The location of the mos genes in the bacteria extends the opine concept, initially developed for a plant pathological interaction, to a symbiotic one.

RHIZOBIUM MELILOTI INSERTION ELEMENT ISRm2 AND ITS USE FOR IDENTIFICATION OF THE fixX GENE

/I.Dusha, S.Kovalenko, Zs.Banfalvi and A.Kondorosi/

Journal of Bacteriology, 1987. Apr. p. 1403-1409.

Two of the three plasmids of the wild-type Rhizobium meliloti 41 /pRme41a and pRme41c/ carry a copy of ISRm2, a 2.7-kilobase-long transposable element. ISRm2 is terminated by 22-base-pair /bp/ inverted repeat sequences, exhibiting some homology to the inverted repeats of elements generating 9-bp target sequence duplication. Transposition of ISRm2 results in a duplication of 8bp in length, rather rare among transposable elements. DNA sequences homologous to an internal fragment of ISRm2 were found in several Rhizobium species. Transposition of ISRm2 into fixation and nodulation genes located on the symbiotic plasmid pRme41b was detected at a high frequency. Exact locations of two copies of ISRm2 which transposed into the nod-nif region on the megaplasmid were determined. In one case, integration into the protein-coding region of the hsnD gene that determines a host specificity function of nodulation occurred. In the other mutant, ISRm2 was localized upstream of nifA, where a short open reading frame coding for a new fix gene /fixX/ was identified. The product of fixX is a ferredoxin carrying a characteristic cluster of cysteine residues. On the basis of the observation that the arrangement of the ISRm2 copies is identical in the free-living wild-type cells and in nitrogen-fixing nodules, we concluded that the involvement of ISRm2 transposition in the development of nitrogen-fixing symbiosis is unlikely.

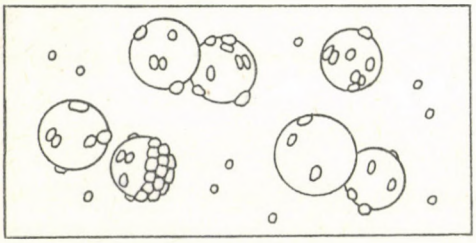
HOST-SPECIFIC REGULATION OF NODULATION GENES IN RHIZOBIUM IS MEDIATED BY A PLANT-SIGNAL, INTERACTING WITH THE nodD GENE PRODUCT

/B.Horváth, C.W.B.Bachem, J.Schell and A.Kondorosi/

The EMBO Journal, 1987. Vol.6. No.4. p. 841-848.

We have identified a nodD gene from the wide host-range Rhizobium strain MPIK 3030 /termed nodD1/ which is essential for nodulation on Macroptilium atropurpureum /siratro/. Experiments with nodA-lacZ gene fusions demonstrate that the MPIK3030 nodD1 regulates expression of the nodABC genes. Additionally, we used nodC-lacZ fusions of Rhizobium meliloti to show that the MPIK3030 nodD1 gene induces expression of these fusions by interacting with plant factors from siratro and from the non-host Medicago sativa /alfalfa/. The R. meliloti nodD genes, however, only interact with alfalfa exudate. In line with these results, no complementation of MPIK3030 nodD1 mutants could be obtained on siratro with the R. meliloti nodD genes, while the MPIK3030 nodD1 can complement nodD mutants of R. meliloti on alfalfa. Furthermore, R. meliloti transconjugants harbouring the MPIK3030 nodD1 efficiently nodulate the illegitimate host siratro. When compared with other nodD sequences, the amino acid sequence of the MPIK3030

nodD1 shows a conserved amino-terminus, whereas the carboxy-terminus of the putative gene product diverges considerably. Studies on a chimeric MPIK3030/R. meliloti nodD gene indicates that the carboxy-terminal region is responsible for the interaction with plant factor/s/ and may have evolved in different rhizobia specifically to interact with plant-host factors.



NUCLEIC ACIDS RESEARCH
/B.Kiss, Z.Végh and É.Vincze/
1987. Vol.15. No.8. p. 3620.

Nodule poly/A/$^+$ mRNA of Medicago sativa L. Cardinal inoculated with Rhizobium meliloti 41 /AK 631/ was used to construct a cDNA library in a lambda phage vector 1149 /1/. Nodule specific sequences were isolated after differential hybridization using labelled nodule and root poly /A/$^+$ mRNA probes. A cDNA clone, which hybridized also to a Lb cDNA insert /pPsLb101/ from Pisum sativum /2/ was sequenced using the chain termination method /3/. The sequence of 381 bp is presented below. The deduced amino acid sequence showed homology to Lb-s from different legumes /4, 5, 6/ and most resembled LbIII of Medicago sativa /7/.

ELIMINATION OF NON-SPECIFIC NUCLEASES FROM RESTRICTION ENDONUCLEASE PREPARATIONS BY DIFFERENT BINDING ON FREE DNA LIGAND
/P.Geck, A.Molnár and I.Nász/
Acta Microbiologica Hungarica,
1987. Vol.34. No.3-4. p. 241-245.

In the purification of a novel restriction endonuclease /an Ava III isoschizomer, isolated in this laboratory/ standard methods were insufficient to eliminate non-specific nuclease contaminations. Taking advantage of the specific site recognition and binding of the restriction endonuclease on DNAs, a method is described for the simple extraction of non-specific nucleases. DNA substrates without recognizable sites do not bind the restriction endonuclease, while non-specific nucleases are absorbed to, and eliminated with, the DNA via gel filtration chromatography under special conditions.

ADHESION PROPERTIES OF E.COLI CELLS IN THE PRESENCE OF PROMETHANIZE
/J.Molnár, K.Csiszár, E.Czirok and E.Szöllősy/
Zentralblatt für Bakteriologie Mikrobiologie und Hygiene, Series A.
1987. Vol.266. p. 276-283.

Some E.coli strains were tested for adsorption to HEp-2 cells and on aluminium hydroxide gel. The adhesiveness of E. coli to HEp-2 cells was inhibited by promethanize. MRHA /mannose-resistant haemagglutinating activity/ positive plas-

mid carrying E. coli strains were found to be adsorbed to tissue culture cells more effectively than the MRHA-negative strains. Fifty percent of the clinical isolates contained antibiotic resistance plasmids, but only 40 % of these strains were able to transfer the antibiotic resistance properties to E. coli as recipient. It is presumed that the hydrophobic adsorption of bacteria depends on the fimbriae, while aluminium hydroxide gel adsorption correlates with surface properties other than the fimbriae.

GENOME LOCATION AND IDENTIFICATION OF FUNCTIONS DEFECTIVE IN THE BARTHA VACCINE STRAIN OF PSEUDORABIES VIRUS
/B.Lomniczi, S.Watanabe, T.Ben-Porat and A.S.Kaplan/
Journal of Virology, 1987. Mar. p.796-801.

We have shown previously /Lomniczi et al., J.Virol. 52: 198-205, 1984/ that the Bartha vaccine strain of pseudorabies virus has a deletion in the short unique /U_s/ region of its genome - a deletion that is related to the absence of virus virulence. This strain is, however, also defective in other genes involved in virulence. We show here that virulence can be restored by marker rescue of the Bartha strain to which an intact U_s has been restored /but not to the parental Bartha strain/ by sequences derived from approximate map units 0.460 and 0.505 of the wild-type virus genome. No difference in the ability to grow in cell culture was observed between parental Bartha, Bartha 43/25a /Bartha to which an intact U_s has been restored/, or the doubly rescued Bartha strains. However, only the doubly rescued Bartha strain was virulent for both chickens and pigs and replicated to high titers when inoculated directly into the brains of chickens. The sequences that could restore virulence to the Bartha 43/25a strain encode four genes, all of which are involved in process leading to the assembly of nucleocapsids. Since these sequences rescue virulence, it appears that a function that plays a role in nucleocapsid assembly is defective in the Bartha strain and that this defect contributes to the lack of virulence of this virus.

A SPECIES-SPECIFIC DNA PROBE FOR THE DETECTION OF MYCOPLASMA GALLISEPTICUM
/M.Sántha, K.Burg, I.Raskó and L. Stipkovits/
Infection and Immunity, 1987. Nov. p.2857-2859.

An 800-base-pair DNA fragment from a partial genomic library of Mycoplasma gallisepticum was selected and used as a probe for the selective detection of this avian pathogen. The specificity and sensitivity of this probe were demonstrated by using dot blot and Southern hybridizations.

ISOLATION OF HIGH MOLECULAR WEIGHT PLANT NUCLEAR DNA SUITABLE FOR USE IN RECOMBINANT DNA TECHNOLOGY
/T.Kiss, F.Solymosy/
Acta Biochimica et Biophysica

Academiae Scientiarium Hungaricae, 1987. Vol.22. No.1. p. 1-5.

TYR-D-ALA-GLY-/ME/ PHE-CHLOROMETHYL KETONE: A MU SPECIFIC AFFINITY LABEL FOR THE OPIOID RECEPTOR

/S.Benyhe, J.Hepp, J.Simon, A.Borsodi, K.Medzihradszky and M.Wolleman/

Neuropeptides, 1987. Vol.9. p. 225-p. 225-235.

An alkylating tetrapeptide enkephalin derivative, Tyr-D-Ala-Gly-/Me/ Phe-chloromethyl ketone /DAMK/ was synthesized, and its binding characteristics on rat brain membranes were evaluated. In competition experiments, the product shows high affinity for the mu opioid binding site of the rat brain membranes, whereas irs binding to the delta and kappa subtypes is weak. Micromolar concentrations of this ligand produce a dose-dependent, apparantly irreversibe inhibition of /^{3}H/-nalaxone binding with apparent IC_{50} value of 1-5 μM. Neither reversibly binding opioids nor tosyl-amino acid chloromethyl ketones show these effects. Saturation binding analysis with /^{3}H/-nalaxone of membranes preincubated with Tyr-D-Ala-Gly-/Me/ Phe-CH_2Cl reveal a selective and irreversible inhibition of the high affinity /^{3}H/-nalaxone binding site. Irreversible blockade of mu-selective /^{3}H/-ligand binding by Tyr-D-Ala-Gly-/Me/ Phe-CH_2Cl is much more effective than that of the binding of /^{3}H/-enkephalin or /^{3}H/-ethylketocyclazocine. The mu-selective binding properties of this new irreversible enkephalin analogue suggest that it could serve as an affinity label for the mu opioid receptor subtype.

HYBRIDIZATION OF INDUSTRIALLY IMPORTANT ASPERGILLI VIA PROTOPLAST FUSION

/F.Kevei, J.Szamos, A.Hoschke, S.Rish and L.Ferenczy/

Biotechnology and Food Industry, 1987. Paper No.5.

The genus Aspergillus is in general of great importance in various biotechnological processes. Black Aspergilli are well known to include members of use for the industrial production of organic acids and enzymes. The production level of industrial strains can be improved by either mutation or recombination. Repeated mutagenic treatment to enhance production may lead to unfavourable cryptic mutations or result in instability owing to translocation or duplication in certain chromosomal segments. The members of the A.niger group are typical imperfect fungi; genetic recombination may occur during the parasexual cycle. Protoplast fusion is an alternative and very effective method for vegetative hybridization. Following mutagenic treatment /UV, NTG/, possible complementing parental strains /as auxotrophs/ could be isolated from naturally-occurring wild-type producers. Mutants derived from various parental sources in all possible combinations were hybridized via protoplast fusion. Protoplasts can be prepared from surface growing cultures by using Trichoderma enzyme in the presence of an inorganic stabilizer, such as KCl. Fusion experiments were performed by means of 25 % PEG 4000

with 0.1 M $CaCl_2$. From successful crosses, fusion products were recovered under selection pressure. At the beginning of regeneration, they proved to be predominantly heterokaryons. When auxotrophic parental markers were used, prototrophic hybrids /diploids/ were isolated from heterokaryons. In the presence of haploidizing agents, such as benzimidazole derivatives, prototrophic hybrids produce sectors showing parental and recombinant segregation. The whole parasexual cycle will be demonstrated. The practical aim of this work was to select for prototrophic haploid recombinants and screen them for production.

PLOIDY-DEPENDENT SEPARATION OF SACCHAROMYCES CEREVISIAE PROTOPLASTS ON DENSITY GRADIENTS

/Cs. Vágvölgyi, J. Kucsera and L. Ferenczy/

Proceedings 4th European Congress on Biotechnology 1987, Vol.1. p. 506-507.

It is a well known phenomenon that cells of higher ploidy can appear in an originally haploid population of cells. This holds true even for heterothallic cells of identical mating type due to mutations in the mating type locus or self-polyploidization. In populations of wild-type cells or those without proper genetic markers, differentiation between haploids and di/tri/tetraploids is hardly feasible. The selection of cells of a certain ploidy is especially complicated if their proportion to the cells of the dominating ploidy level is extremely low. Though there are differences in size and weight between cells of various ploidy levels, the overlapping is too strong. This is mainly due to the irregular shape of the cells and the presence of the cell wall. If the cell wall is removed, a much better distinction between the different ploidy levels can be achieved.

Model experiments were carried out with well-marked Saccharomyces cerevisiae cells of different ploidy levels /di/tri/tetraploids/ constructed from polyauxotrophic haploid mutants.

For preparations, three different configuration media /Ficoll[1], Nycodenz[2], Percoll[3]/ were used. Both continuous and discontinuous gradients /iso-osmotic throughout/ were checked for the separations. Good results were obtained with continuous gradients of Nycodenz and Ficoll /5-25 %/ osmotically stabilized with 0.6 M KCl by employing rate-zonal configuration. Separations based on the buoyant densities of the protoplasts were examined on different Percoll gradients /45-95 %/. Samples of protoplasts of various ploidy levels were separated, regenerated and analysed /cytologically, biochemically and genetically/. The best separations were also tested with a number of other Saccharomyces cerevisiae strains.

The procedure of ploidy-dependent separation of protoplasts, among others, gives an opportunity to select cell lines of higher ploidy level after mating or after fusion of protoplasts of both identical and opposite mating types, without introducing genetic markers into the partners involved in constructing new industrial

strains. Such density gradient systems are also suitable for the separation of high-ploidy level cells from low-ploidy level ones and vice versa, even if their numbers in a population are extremely different. The separation of anucleate protoplasts from nucleate ones is similarly practicable.

GENE TRANSFER VIA CHEMICALLY INACTIVATED PROTOPLASTS OF YEASTS

/J.Kucsera and L.Ferenczy/

Proceedings 4th European Congress on Biotechnology 1987, Vol.1. p. 508.

In all previous fungal protoplast fusion experiments, genetic methods have been used to select the fusion hybrids, mainly on the basis of the complementation of auxotrophic strains. Unfortunately, this approach seem virtually impracticable with industrial yeast strains. The introduction of applicable selective markers into the fusion partners with higher ploidy level is laborious if not impossible in practice, and at the same time frequently deleterious to the existing genetic composition of industrial strains. On the other hand, without selectable markers it is rather difficult to recognize fusion products. In order to overcome these problems, we have worked out a biochemical method of selection instead of the genetic one.

Antifungal compounds with different modes of action were selected to inactivate protoplasts without altering their genetic composition or lysing them. The basic idea was to find fungicides which inhibit certain basic enzymatic processes of the cells, e.g. by irreversibly inactivating enzymes of vital importance. Their application may lead to a comparatively rapid cell death, except that the inactivated enzymes are replaced by functioning ones in time. Transfer of functioning enzymes, i.e. cell reactivation, can be attained by the fusion of protoplasts /"enzyme transfusion"/. Under appropriate conditions, only the fusion hybrids will yield colonies. Model experiments were carried out with Saccharomyces cerevisiae strains. Stable auxotrophic mutants were used to check the applicability of the biochemical method of selection: protoplasts were obtained by snail enzyme treatment from cells, and were treated with different concentrations of fungicidal compounds for various periods. Of the compounds tested, N- ethylmalimide /50 μg/ml, 60 min/ and miconazole /10 μg/ml, 30 min/ proved to be most efficient. Both inhibitors yielded 100 % inactivation even at high concentrations of protoplasts; at the same time effective reactivation and hybrid formation resulted after fusion with untreated, complementing auxotropic protoplasts.

BIOCHEMICAL ALTERNATIVE TO MUTAGENESIS: MODEL EXPERIMENTS WITH AUXOTROPHIC STRAINS OF ASPERGILLUS NIDULANS

/L.Ferenczy, R.E.Bradshaw, F.Kevei and J.F.Peberdy/

Proceedings 4th European Congress on Biotechnology 1987, Vol.1. p.

In fungal protoplast fusion experiments, genetic methods are used to select the fusion hybrids. In most cases the selection

is based upon the complementation of auxotrophic strains. However, this approach is unsuccessful with many industrial strains of yeasts and filamentous fungi, due to the higher ploidy level or to the fact that the introduction of auxotrophy normally leads to a dramatic decrease in productivity.

The basic idea was to find antifungal compounds which can irreversibly inactivate enzymes of vital importance. Their application may lead to rapid cell death. However, cell reactivation can be attained by transfer of functioning enzymes via protoplasts fusion /"enzyme transfusion"/. Under appropriate conditions, only fusion hybrids will yield colonies.

Model experiments were carried out with Aspergillus nidulans 1156 /ade$^-$ PABA$^-$y/ and 1157 /lys$^-$ PABA$^-$ y/ to check the applicability of the biochemical method of selection. A series of antifungal compounds with different modes of action were employed. Protoplasts were formed and treated with various concentrations of the different compounds for different times. The compounds were removed, and the protoplasts were checked for integrity, inactivation, and reactivation by fusion. The most efficient compounds were: Crystal violet, N-ethylmaleimide, 8-hydroxyquinoline, malachite green, miconazole, oligomycin, pyrrolnitrin.

In principle, all fungicidal compounds can be used which

/a/ inactivate basic enzyme reactions rapidly and uniformly, and

/b/ are removable if not reacted, i.e. molecules taken up but remaining free will not poison the untreated protoplasts after fusion;

/c/ do not disintegrate protoplasts;

/d/ do not alter the genetic composition of the cells.

ELECTROFUSION OF ASPERGILLUS NIDULANS PROTOPLASTS

/A.Frankó, L.Ferenczy/

10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Electric field-induced fusion was carried out between two auxotrophic mutants /ade$^-$ and lys$^-$/ of Aspergillus nidulans. Close membran contact between the protoplasts was achieved by dielectrophoresis in an inhomogeneous alternating field /0,8kV/cm field strength and 10 s duration/. Due to dielectrophoresis pearl chains of two strains are formed between the electrodes. Protoplast fusion was induced by application of a single square field pulse sufficiently high to induce reversibly breakdown in the membrane contact zone between cells within a pearl chain /3,3 kV/cm, 40 μs/. Genetic complementation of the auxotrophic strains indicated the occurrence of fusion. In the case of electrofusion 7 to 20 times more heterokaryotic colonies were grown as compared to the fusion.

PRODUCTION OF NEOCOMBINANTS BY PROTOPLAST FUSION OF CLAVICEPS PURPUREA STRAINS

/Á.Nagy, L.Manczinger, K.Zalai and L.Ferenczy/

10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Protoplast fusion can be used as an efficient tool for the combination of genoms of strains, mainly industrial strains, which do not have normal sexual process. The fusion was induced by PEG, with auxotrophic mutants of the parental strains, and the hybrids were selected on minimal medium.
To decrease the possibility of back mutation the fusion technique was carried out with different diauxotrophic Claviceps purpurea strains.
To characterise these fusion products, classical genetic analyses were used. Several examinations were carried out to prove whether or not caryogamia took place. Uninuclear conidia were germinated on minimal medium. The content of DNA/nucleus was measured with different fluorescence compounds, DABA and DAPI. Recombinants and neocombinants were obtained by using MBC as haploidization agent.

CHARACTERISATION OF THE FREE-LIVING AMOEBAS ISOLATED FROM PATHOLOGICAL SAMPLES AND THE NATURE

/A.Matyi, A.Prókai, É.Tóth and J. Földes/
10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Acanthamoeba castellanii and Acanthamoeba polyphaga were isolated from pathological samples of meningoencephalitis and ulcerativ keratitis. Many other free-living amoebas were isolated from the swimming-pool, the river and the soil of the area.

The isolated amoebas were characterized by the morphological examination of the trophozoites and cysts, the pathogenicity investigated in mice, the antigenic structure analysed by serological methods and isoenzym profile compared with PAGE. The morphological-, biological- and serological markers proved the phylogenetic relations between the free-living amoebas isolated from the pathological samples of the milieu.

DETECTION OF FOUR SPECIES WITHIN THE TRICHODERMA HARZIANUM AGGREGATE BY PROTOPLAST FUSION

/L.Manczinger and B.Furka/
10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

The ability of 65 Trichoderma isolates to grow on 127 carbon sources was tested. Cluster analysis was performed on the basis of the data derived from the utilization of the 42 carbon sources which gave reproducible results. Four groups of Trichoderma harzianum could be clearly identified.
To prove that these groups are indeed distinct species an isolate of each groups was selected and their auxotrophic protoplasts were fused by the PEG-Ca^{++} method in pairwise combinations. No heterokaryons were obtained between the groups, but vigor heterokaryons appeared within a strain if the fusions were made with the same protoplast suspensions. The above results strongly support the idea that at least four species exist within the Trichoderma harzianum spe-

cies aggregate.

A NEW METHOD TO INCREASE THE REGENERATION FREQUENCY OF ACREMONIUM PROTOPLASTS

/J.Varga and L.Ferenczy/
10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Protoplasts of some industrially important Acremonium chrysogenum strains have very poor regeneration rates in the universally used agar media. The application of solidifying macromolecules other than agar may increase the regeneration frequencies of these protoplasts. Cryoprecipitate, agarose and Ca-alginate were tested. Cryoprecipitate is a freeze-dryed fraction of blood-plasma which contains mainly proteins. It's clotting may be induced by adding Ca-ions. In the regeneration experiments protoplasts were embedded in 1 % agarose or Ca-alginate, or in 5-10 % cryoprecipitate supplemented with 0,5 % $CaCl_2$, and plated onto a bottom layer. Osmotic stabilization was carried out by using non-ionic stabilizer such as sucrose, because the high ionic strength of the most frequently used ionic stabilizers prevents blood coagulation as well as gelation of cryoprecipitate.
Using agarose or Ca-alginate, 1,5-2 times better regeneration rates were obtained than in the case of agar as a solidifying agent. The best results were reached with cryoprecipitate. Two-four times more regenerating colonies developed in this medium than on the control plates.

FUSION OF CHEMICALLY INACTIVATED YEAST PROTOPLASTS

/J.Kucsera and L.Ferenczy/
10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Protoplasts of Saccharomyces cerevisiae were inactivated with different concentrations of antifungal compounds for various periods. Of the 24 compounds tested N-ethylmaleimide proved to be the most efficient: at a concentration of 50 μg/ml for 30 min caused 100 % inactivation at high concentration of protoplasts, without altering their genetic composition or lysing them. The inactivation effect is fully reproducible.
Such inactivated protoplasts could function as fusion partners. When they were fused with normal /untreated/ protoplasts by polyethylene glycol treatment, they produced viable hybrid cells. The analysis of the fusion product from inactivation experiment showed similar result to the control /uninactivated/ fusion products.
The chemical inactivation method seems to provide a new way to counterselect fusion hybrids when the introduction of selective genetic markers /f.e. auxotrophic mutation/ is impossible or when it can be deletorious to the existing genetic composition of industrial strains.

BLACK ASPERGILLI: MICROBES OF GREAT BIOTECHNOLOGICAL IMPORTANCE

/F.Kevei/

10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged/

Filamentous fungi are in general of great importance in various biotechnological processes. The genus Aspergillus is well known to include members of use for the industrial production of organic acids and enzymes.

Black Aspergilli are typical imperfect fungi. This report will discuss problems of conventional and new breeding procedures of these species, such as mutagenesis, recombinant selection following parasexual crosses, protoplast fusion and the possible applications of recombinant DNA techniques.

CHARACTERIZATION OF INTERSPECIFIC HYBRID OF TAXONOMICALLY DISTANT ASPERGILLII BY ISOENZYME ANALYSIS

/É.Tóth and L.Ferenczy/

10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

The interspecific hybrid was obtained by fusing the protoplasts of auxotrophic mutants of Aspergillus nidulans and Aspergillus fumigatus with polyethylene glycol. Following the genetic analysis the hybrid was characterized by isoenzyme analysis. The liquid minimal medium was inoculated with conidia suspension of the parental strains or mycelia suspension of the hybrid. In order to avoid the effect of the catabolit repression in case of some enzymes the glucose was substituted for Na-acetate, the ammonium-ion for nitrate-ion, "Skim milk powder", uric acid. After a three day of cultivation the mycelia was harvested, freezed-thawed inthree cycles and ground in a mortar. The cell-free extract was analysed by gel electrophoresis. Nineteen kinds of enzymes were examined. Our results suggested that the hybrids contain the whole A.nidulans genome. In a few cases A.fumigatus isoenzymes and new allelic forms appeared. These proved that the nuclear fusion took place.

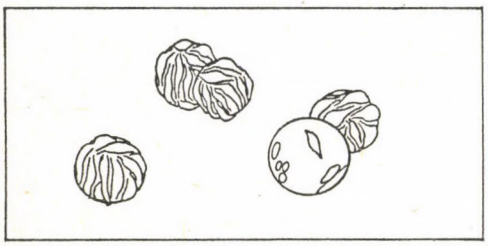
CENTRIFUGAL SEPARATION OF SACCHAROMYCES CEREVISIAE PROTOPLASTS WITH DIFFERENT PLOIDY LEVELS

/Cs.Vágvölgyi, J.Kucsera and L. Ferenczy/

10the Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Attempts have been made to find methods suitable for centrifugal separation of Saccharomyces cerevisiae protoplasts of different ploidy levels. Model experiments were carried out with well marked Saccharomyces cerevisiae strains /di/tri-/tetraploids/ constructed from polyauxotrophic haploid mutants. For preparations three different centrifugation media /Ficoll, Percoll, Nycodenz/ were used. Good results were obtained with continu-

ous gradients of Ficoll and Nycodenz /5-25 %/ by employing rate zonal centrifugation. Separations based on the buoyant densities of the protoplasts were examined on different Percoll gradients /45--95 %/. Samples of protoplasts of various ploidy levels were separated, regenerated and analysed. The procedure of ploidy-dependent separation of protoplasts, in certain cases, gives an opportunity to enrich cells of higher ploidy after fusion or after mating, without introducing genetic markers into the partners.



DELETION OF VECTOR DNA SEQUENCES IN CLONING OF A 2μ DERIVATE PLASMID OF S.CEREVISIAE RXII

/K.Büttner, L.Ferenczy and M.Mink/

10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Systematic study of the mitochondrial DNA /mtDNA/ of the S.Cerevisiae strain RXII revealed that the 10000 g membraneous-mitochondrinal pellet, the source of mtDNA, contained two other extrachromosomal genetic elements. One of them was resolved as a 6.2 kilobasepairs /kbp/EcoRI fragment in agarose gel electrophoresis and hybridized to the part of a 2μ probe.

The 6.2 kbp band was isolated from the gel, ligated into the EcoRI site of the pBR328 vector and the E.coli HB101 strain was transformed. The restriction mapping of the chloramphenicol sensitive plasmids showed that the vector sequences, between the ampicillin and tetracyclin resistance gene promoters were deleted. This event was not detected when EcoRI linearized vector DNA was ligated and transformed: all of the clones recovered were chlorampenicol resistant. These data suggested that cloning of the entire 2μ derivative DNA of strain RXII gave room for expression of gene/s/ encoded by the inserted DNA, which resulted in a such a hars recombination event in a recA13 background.

HYBRIDIZATION AND BREEDING OF GLYCOAMYLASE PRODUCER ASPERGILLUS NIGER STRAINS VIA PROTOPLAST FUSION

/F.Kevei, S.Risch, J.Szamos and A. Hoschke/

10th Congress of the Hungarian Society of Microbiology, 26-29 August 1987 Szeged

Glycoamylase producer A.niger strains derived from various sources possess interesting common characteristics which have alternative proline or arginine requirements. Following mutagenic treatment /UV, NTG/ single proline or arginine requireing strains could be isolated, but these auxotrophic markers were not complementing in attempted crosses.

Strains carrying additional nutritional

requirements, like cysteine, leucine, lysine, adenine, cytosine, PABA were also isolated. These markers proved to be complementing characters in crosses. Auxotrophic mutants derived from various parental sources in all possible combinations were hybridized via protoplast fusion. Protoplasts can be released from surface growing cultures by using induced Trichoderma-enzyme. Fusion experiments were performed by 25 % PEG 4000 with o,1 M $CaCl_2$. From all attempted crosses fusion products were recovered. At the beginning of regeneration they proved to be heterokaryons. Prototrophic hybrids - possible diploids - were isolated from the heterokaryons. Using benzimidazole derivatives as haploidizing agents prototrophic hybrids produced sectors showing parental and recombinant segregation. The stability of parental-, hybrid-, and haploidized clones is discussed. We demonstrate here the whole parasexual cycle. The practical aim of this work is to select for prototrophic haploid recombinants and screen them for glycoamylase production in submerged condition.

SERVICES FOR BIOTECHNOLOGY: MICROBIAL STRAIN DATABANK AND INDUSTRIAL PATENT DEPOSITARY

/T.Török, J.Lehoczky and T.Deák/

Within the frame of the Hungarian biotechnology program the National Collection of Agricultural and Industrial Microorganisms /NCAIM/ has recently received substantial financial support for further development of its services for research and industrial applications in the field of biotechnology.

Culture collections are important to biotechnology for a variety of reasons. Their prime function is to provide a wide range of pure and authentic microorganisms that are of past, present or potential interest. Culture collections not only accumulate a valuable pool of genetic resources in the form of microbial strains but by applying proper methods of preservation they also prevent loss or change of these strains. In the course of routine work with cultures, physiological and biochemical tests are performed with each strain, accumulating a large number of data. Based on this information it can be predicted which strain may be useful to programmes in biochemistry, genetics and biotechnology. Frequently, this kind of information can be of equal importance to those of microbial strains themselves. Computers offer reliable and fast way to record, store and retrieve the large amount of data which a culture collection has to comply with. Computers serve not only the day to day task of running a collection by updating records, controlling stock and printing a catalogue, but they also offer means to search the database for strains with particular characters and required properties.

Databanks based on information available in culture collections have been established in a number of countries. Efforts have been made to develop an international network of databases. Notable are the Microbial Strain Data Network /MSDN/ under the auspices of the World Federation of Culture Collections /WFCC/

and the Microbial Information Network Europe /MINE/.

In order to keep up with the up-to-date requirements we started to develop a computerized database of NCAIM, based on an IBM AT personal computer. Table 1 shows the menu of the program. Data input can be made under the following headings for each strain: nomenclature, origin, identification, reference to other collections, literature, special features and usage, maintenance, source. Preparations are being made to join the national network of databases and to international databanks.

A further important service to biotechnology is offered by culture collection as depositories for patent strains. The majority of biotechnological inventions are directly connected to a specific strain of micro-organism. The patent laws of most countries require that the strain involved in a patent application must be deposited with a culture collection.

In 1977 an international agreement called Budapest Treaty was reached which allows a single application to be recognized in a number of contracting countries /Table 2/. The major advantage of the Treaty is that a single culture deposit in an approved collection will satisfy the requirements of patent application and provide legal protection to inventors in all member countries.

In order to qualify as an approved international depository authority, a culture collection must meet certain requirements /Table 3/, of which the most important are stability, facilities and scientific competence. At present there are 14 international depository authorities, the last one NCAIM was approved in July, 1986 /Table 4/.

At NCAIM we follow the basic protocol for a deposit under the Budapest Treaty as it is specified in its statutes. The depositor must state in a letter accompanying the culture that the strain is being deposited under the Budapest Treaty. After the preservation procedure which is made mostly by freeze-drying, the collection performs a viability test and fills out a form that includes the accession number of the strain. It is returned to the depositor who is responsible for completing the filling of patent application. Once the patent has been issued, the respective strain must be stored at the culture collection for at least 30 years. A culture is available to other persons according to the rules of the Treaty. It is important to note that a decision on release of a culture is made by the respective patent office and not by the depository authority.

In addition to the activities mentioned above, the NCAIM offers several other services /Table 5/ for the benefit of workers interested in microbiology, genetics, biochemistry and other fields connected to biotechnology.

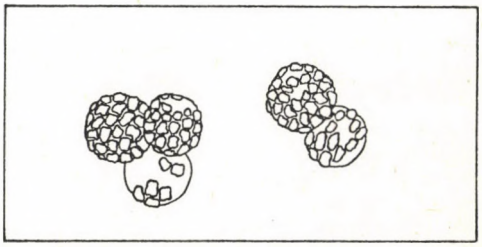


Table 1. Menu of the computer program of NCAIM

National collection of agricultural and industrial microorganisms
1/ New strain data input
2/ Edit data
3/ Delete data
4/ Print strain data
5/ Strain distribution
6/ Monitoring
7/ Catalogue
0/ Exit main menu
Press restricted key. /0-7/
If selected function is o.k., then press ENTER

Table 2. Member states of the Budapest Treaty at January 1, 1987

Austria	1985	Japan	1980
Belgium	1983	Liechtenstein	1981
Bulgaria	1980	Norway	1986
Denmark	1985	Philippines	1981
Finnland	1981	Soviet Union	1981
France	1980	Spain	1981
Fed.Rep.Germany	1981	Sweden	1983
Hungary	1980	Switzerland	1981
Italy	1986	United Kingdom	1980
	USA 1980		

Table 3. Requirements and tasks of depository authorities

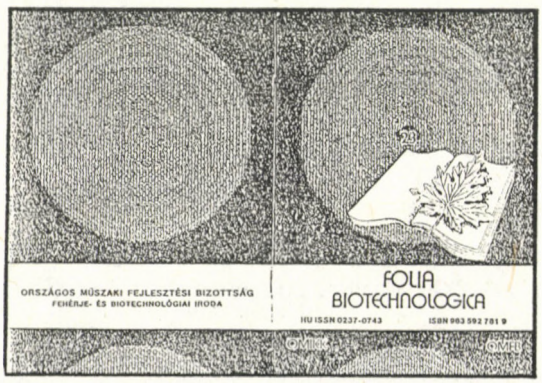
1. Continuous existence
2. Necessary staff and facilities
3. Accept microorganisms for deposit, examine their viability
4. Storage of deposits for a period of 30 years
5. According to specified and regulated conditions
   - comply with security and secrecy of deposits
   - issue documents about them
   - furnish samples to parties legally entitled

Table 4. International depository authorities for patent strains of microorganisms

1. ATCC	American Type Culture Collection USA	1980
2. ARCC	Agricultural Research Culture Collection USA	1980
3. FRI	Fermentation Research Institute Japan	1981
4. DSM	Deutsche Sammlung von Mikroorganismen Fed.Rep.Germany	1981
5. CBS	Centralbureau voor Scimmelcultures Netherland	1981
6. NCTC	National Type Culture Collection UK	1982
7. NCYC	National Collection of Yeast Cultures UK	1982
8. NCIB	National Collection of Industrial Bact. UK	1982
9. CCAP	Culture Centre for Algae and Protozoa UK	1982
10. CMICC	Cult.Coll. Commonwealth Mycological Institute UK	1983
11. IVI	In Vitro International, Inc. USA	1983
12. CNCM	Coll. Natl. de Cultures de Microorg. France	1984
13. ECACC	European Collection of Animal Cell Cultures UK	1985
14. NCAIM	National Collection of Agricultural Indust. Microorg. Hung.	1986

Table 5. Services offered by NCAIM

1. Deposits
   - Patent deposition under the BT
   - Hungarian patent deposit
   - Safe deposit
2. Preservation
   - 20 freeze dried ampoulles
   - 6 vials frozen under liquid N
3. Identification
   - Yeasts
   - Aerobic spore-forming bacilli
   - Pseudomonads and enterobacteria
   - Lactobacilli
4. Training
   - Preservation and maintenance
   - Identification
5. Other services
   - Consultation
   - Off-line computer service on strain data and availability



GENETICS /Textbook/
/László Heszky, József Sutka/
GATE, 1987

Contents

IN VITRO RECOMBINATION /Genetic Engineering/
/L.Heszky and J.Sutka/
"Genetika", Chapter VII, p. 118-138, GATE, 1987.

The in vitro recombination means the artificial transfer and ligation of different DNA fragments by means of a highly sophisticated molecular technology, in contrast to the in vivo recombination process. The genetic engineering is a new profitable strategy in biotechnology. The genetic information determining the phenotype of the living organism is cut into smaller fragments carrying given information and those information packages can be transferred from one organism to the other. The economic importance of this technology is based on the fact that the difference species can develop new advantageous characteristics and can produce programmed substances, like drugs, diagnostics, monoclonals, etc. The in vitro DNA recombination, therefore, creates new combinations of the genetic material by means of the following complicated molecular genetic and cytogenetic steps: DNA is fragmented with restriction endonucleases, the fragments are amplified /cloning, clone-libraries/, the given gene is identified /clone-selection, hybridization/, the gene is inserted to the transfer systems /vectors/, the transferred gene is integrated into the recipient's genom, it should be replicated, the gene should be inherited. The Chapter covers the details of these steps and gives examples of plant and animal cell transformation as well as of the recombination with artificially synthesized genes.

THE MOLECULAR BASES OF IMMUNOGENETICS
/L.Heszky and J.Sutka/
"Genetika", Chapter VIII, p.139-150, GATE, 1987.

The constituents of the immune-system can be divided into three major groups in in a simplified way: B-cells, T-cells, and MHC proteins. The function of the immune-system is to recognize then eliminate the antigene. Since there is an almost infinite number of antigenes, the genetic problem is how the organism can produce such a great variability of antibodies, antigene-receptors, and MHC proteins. The genetic variability of these proteins is explained on the model of immunoglobuline-genes. The antibody /immunoglobuline/ molecule consists of two light and two heavy chains. Both the light and the heavy chains can be divided into constant and variable domains. The antigene-binding site is located at the end of the variable domain. The genes of the antibody molecule are scattered in the genom and the synthesis of the light or heavy chain is preceded by a gene-rearrangement which joins the V /variable/, J /joining/, D /diversity/, and C /constant/ genes into one functioning unit. There are a given number of V, J, D and C genes each and their combinations represent the main source of the diversity of antibodies. Another possibility is the somatic mutation which also contributes to the high number of antibodies present in

the organisms. A given antibody is produced by one B-cell clone only thus, its amount is limited. Antibodies specific for one antigene can be produced in greater amounts by the hybridoma technique. The production and use of these monoclonal antibodies are discussed in detail.

THE BIOTECHNIQUES OF REPRODUCTION
/L.Heszky and J.Sutka/
"Genetika" Chapter IX, Part 4, p. 182-186, GATE, 1987.

Genetic manipulation for influencing the production of plants and animals is economically important. Both sperms and ova can be manipulated by androgenesis or gynogenesis. These techniques provide to determine the sex of the offspring and the production of extremely productive haploids, e.g. in cases of fishes, sugar-beets, cucumbers, etc. The in vitro fertilization technique offers the possibility for producing hybrids incompatible in vivo due to the action of natural preventing factors. The fertilized ovum can be developed in vitro in case of plants while in animals it should be reinplanted to the female. The zygote transplantation is the principle technique for the animal breeding by genetic manipulation. It offers the possibility of conservation of different genotypes in gene banks and the refrigerated embryos can be applied at any time and sex-manipulated befor their further development. Embryo-manipulation is also possible in plants by an enzymatic treatment followed by the somatic embryogenesis. The development of meristem is used for the cloning of plants, cultivating of protoplasts, etc. The zygote can be chopped into pieces and the regenerated parts are reproduced successfully after transplanting them into a female. Hybrids are produced by the parasexual hybridization techniques using different fusion methods.

THE BASIS OF CYTOGENETICS IN PLANTS
/L.Heszky and J.Sutka/
"Genetika" Chapter X, Part 4, p. 245-252, GATE, 1987.

Plant cells and tissues can be cultured and manipulated in vitro under well-controlled climatized conditions in test tubes. The most common techniques involve somatic or haploid cells or protoplasts. Culturing of somatic cells is based on the production of callus on the culture medium and by means of several inoculations of the primary callus /passage/ can be developed continuously. The production of biologically active materials /i.e. alkaloids/ is one of the main fields of the application of this technique. Callus and cell cultures are also used for the selection of mutants because some millions of embryos can be tested under laboratory conditions. The somaclonal variability explains the production of plants from somatic cultures with altered characteristics. The selection of mutants is more effective by using

haploid cell cultures and pollen cultures. The real genetic manipulation of plant cells is carried on protoplasts. Protoplasts can be isolated both from somatic and haploid cells and heterocaryons can be produced by the fusion of protoplasts. The production of hetero- and homocaryons has economic and industrial importance.

FOLIA BIOTECHNOLOGICA issues published in 1987

FOLIA BIOTECHNOLOGICA Nº 15. 1987.

PARTHENOGENESIS

Reproduction by parthenogenesis in the nature occurs among vertebrates of low order, moreover, it can be provoked artificially, too. However, excluding the literatury, religious and historical myths there is no sign showing that there was ever born by parthenogenesis a man or a mammalia. For the moment there is no example for his experimental provoking. Despite, in the last years many research laboratories of the world started to investigate the mammalian parthenogenesis. The probable reward in the near future is not small. The solution of reproduction of this type at the mammalia will revolutionize the stockbreeding.

FOLIA BIOTECHNOLOGICA Nº 16. 1987.

POSSIBILITIES FOR PRACTICAL APPLICATION OF GROWTH HORMONE IN STOCKBREEDING

All the world follows with interest the investigations on the practical use in stockbreeding of growth hormone produced by recombinant technique. Repeated dosing of hormone for fatted animal stimulates the growth pace, and increases the production of milk in cow. There were succesfull experiments in increasing of animal hormone level by application of special biotechnological methods instead of continuous dosing of growth hormone. This monograph presents the main results of experiments and the possibility of their practical application.

FOLIA BIOTECHNOLOGICA Nº 17. 1987.

GENETIC ENGINEERING IN HIGH ORDER PLANTS

The relation of plant biotechnology and genetic manipulation, the objects of genetic engineering: the plant cell and DNA are discussed. The possible ways of plant regeneration from cultivated plant cell are presented. Before the detailed discussion of molecular methods the common and different features and perspectives of in vivo and in vitro recombination are treated. There is a short review of cloning, fond of clones, selection of clones, main steps of cDNA-synthesis and preparation of synthetic DNA. The Ti-plasmid and the natural transformation vectors, based on CaMV are illustrated, completed by a survey of some new bacterial vector constructions. From the genetransfer systems the infection of seedlings, co-culture, PEG-treatment, electroporation and microinjection are shown. It is followed by an evalution of genetical, immunological, elelctrophoretical and molecular hybridization technics, applicable for proofing of succesfull plant transformation. After presenting new succesfull plant transformations and transgenic plants the perspectives and limits of plant genetic engineering are demonstrated. The work which is complemented by 11 figures and 1 table is very useful for researchers, professors interested in plant genetics, selection and plant biotechnology and as well for students specialized in this area of sciences.

FOLIA BIOTECHNOLOGICA Nº 18. 1987.

The potential of infection elimination in livestock by embryo transfer

Embryo transfer is one of the biotechnical processes gaining more and more ground in everyday practice. The well-known technology – transplantation of an ovum, germ-cell or pre-embryo of a dam to the uterus of an other one – raises two questions:

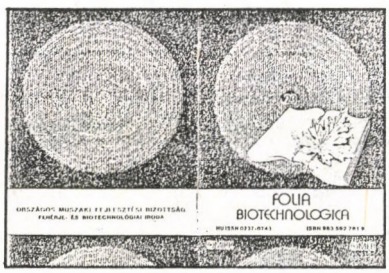
- in what measure can be the embryos and these processes carriers of different infective agents, what role can they play in the transmission of the infectious diseases or
- when the embryo is free of pathogen of a disease, can the embryo transfer be applicated for stopping the infection chain.

If there is a positive first answer then the embryo transfer can not be realized or only with the observance of severe restrictions. If the second response is affirmative, the advantages of embryo transfer (increase of the number of descendants, propagation of entities with valuable genetic material, enhancement of selection intensity, early drawing into breeding, reproduction of descendants from entities not mature) can be completed with the elimination of the infections, or reproducing of a livestock free of certain infectious disease.

FOLIA BIOTECHNOLOGICA Nº 19. 1987.

Utilization of new biotechnology in mammalian livestock production

New biotechnology is a process which starts with the controlled transformation of the genetic material – DNA. As the modification of genome is possible only in the germ-cell, zygote or blastomers of early embryos in cells of mammals, specialists must develop techniques for obtaining these cells, then transfer them into a medium (for the moment into an uterus) where a complete foetus can develop. From biotechniques, opening new perspectives in livestock production this study presents the embryotransfer, micromanipulations and the application of recombinant DNA technique.



DISTRIBUTION PATTERN OF ADENOVIRUS HEXON EPITOPES IN INEFFECTED CELLS DETERMINED WITH MONOCLONAL ANTIBODIES BY IMMUNOFLUORESCENCE ANALYSIS

/É.Ádám, P.Dán, I.Nász, A.Lengyel, J.Erdei and J.Fachet/
Acta Microbiologica Hungarica, 1987. Vol.34. No.3-4. p.247-254.

Two monoclonal antibodies /MAbs/ specific for two distinct epitopes on the human adenovirus type 1 /AV1/ hexon were used to determine the subcellular localization of hexon epitopes in the infected HEp-2 cells by indirect immunofluorescence. On the basis of crossreactivity pattern of MAbs, presumably one of the epitopes in genus specific and the other should be intertype specific. The epitopes, i.e. the adenovirus hexons could be detected throughout the cell and could display different accumulation forms. Fluorescence appeared either in the cytoplasm only or both in the nucleus and the cytoplasm. In the cytoplasm the hexons could be found in diffuse or perinuclear distribution or accumulated into discrete spots. In the nucleus they formed granules or clusters or were diffusely distributed causing a bright fluorescence of the whole nucleus. The different accumulation forms appeared at the same time in different cells of a culture, but in one given cell the fluorescence always appeared first in the cytoplasm.

ISOLATION AND CHARACTERIZATION OF STREPTOMYCIN RESISTANT FERN MUTANTS IN VITRO

/A.Breznovits, Gy.Csanádi, E.Sheffield and G.Vida/
1st Hungarian Genetics Conference, 27-28 April 1987, Budapest, p.3.

Streptomycin resistant cell lines were isolated from primary callus cultures of gametophytic origin of Pteridium aquilinum, Pteris henryi and Pteris cretica cv. albolineata. The method described for tobacco and moss cultures was applied. Frequency of spontaneous mutation was 10^{-6} and selection of streptomycin resistant mutants happened on media containing 1.0 mg ml^{-1} streptomycin sulphate. PSr1, PSr2 and PSr3 lines isolated from Pteridium aquilinum were investigated so far. Mutant callus retained resistance even after several-month of cultivation on drug-free media. Plant regeneration from resistant cell lines was not successful so far therefore we investigate the

transmission of the trait by electrofusion of protoplasts. Electrofusion experiments are performed on a home-made electrofusion equipment. Streptomycin resistance is a result of the insensibility of the mutant chloroplasts according to our TEM /transmission electronmicroscopic/ investigation. Further studies on the investigation of the mutants are in progress.

GAMMA-INTERFERON IS PRODUCED BY CD3+ AND CD3- LYMPHOCYTES

/S.Sandvig, T-Laskay, J.Andersson, M. De Ley and U.Andersson/
Immunological Review, 1987. No.97. p.51-65.

We believe that one useful application of this method in the future will be to detect cytokine producing cells which have been activated in vivo in order to directly assess cell-mediated immunity. We describe IFN-γ synthesizing MNC in this review, but we have also successfully modified the technique to discover cells producing other lymphokines and monokines /unpublished/. The morphological appearence of the extremely rare in vivo activated IFN-γ producing cells in fresh blood samples is identical to that of in vitro induced IFN-γ positive MNC.

It would be an obvious advantage if one could use flow cytometry instead of fluorescence microscopy in order to quantitate the results, saving time and labor. The problem that we have encountered in approximately half of the experiments is that the cells tend to aggregate after the acetone or NP-40 detergent treatment. However, in many experiments, no cell clumping occured and we found excellent agreement between the results obtained with flow cytometry and the naked eye. So far, we have not been able to control the problems of cell aggregation, but this obstacle should not be insurmountable. It should also be emphasized that there has been no problem detecting single IFN-γ positive MNC in cell clumps using the microscope since the immunfluorescent staining has such a characteristic morphology, clearly distinguishing it from the unspecific background staining frequently occurring in cell clumps.

The unexpected finding that approximetely half of the OKT3 induced IFN-γ producing MNC were actually CD3 negative cells could be explained by an early IL-2 secretion in the cultures stimulating CD3 negative, and possibly also CD3 positive, cells to produce IFN-γ . It has previously been reported /Grabstein et al. 1986/ that the second peak accumulation of mRNA for IL-2 occurred already 5 h after polyclonal T-cell activation and clearly preceded that of IFN-γ mRNA by approximately 12 h. It is likely that this early endogenous IL-2 production is of major importance for subsequent IFN-γ synthesis. However, we believe all IFN-γ production cannot be secondary to IL-2 production since IFN-γ synthesizing MNC were evident already 1 h after iniation of the cultures. The results seen in the cord blood cultures demonstrated that IL-2 production and uptake is not always sufficient for IFN-γ synthesis to occur.

We have previously speculated that the reasons for CD3 negative IFN-γ producing cells could be due to internalization or capping and shedding of the CD3-Ti

complex after OKT3 exposure, but later experiments have convinced us that these phenomena only influence our results to a marginal extent. The consistency of the phenotype of the IFN-γ positive MNC appearing 6, 24 and 48 h after OKT3 stimulation, and the congruent results seen after rIL-2 and OKT3 activation, are the main arguments for this view.

Apart from the CD4+CD3+ T-cells and the CD8+CD3+ T-cells, which constituted about half of the IFN-γ producing population, we found that approximately 25 % of the IFN-γ producing cells carried IL-2 receptors and the majority were E-rosetting MNC, but they did not fulfill criteria for either T-cell. B-cell or monocyte cell lineages. Future experiments with simultaneous staining of several different surface antigens will be needed to further characterize these cells.

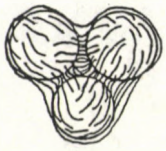
IFN- IS NOT THE ONLY MEDIATOR OF SUPPRESSED MYELOPOESIS PRODUCED BY MONONUCLEAR CELLS FROM APLASTIC ANEMIA PATIENTS

/T.Laskay, M.Hansson, A.Porwit, M. Björkholm, W.Berthold and R.Kiessling/ Journal of Biological Regulators and Homeostatic Agents, 1987. Vol.1. No.1. p.37-44.

Peripheral blood mononuclear cells /PBMC/ from patients, with aplastic anemia /AA/ and healthy donors were compared with regard to their ability to produce soluble factors with inhibitory activity on in vitro granulopoiesis /GM-CFC/.

Although PBMC from AA patients produced enhanced levels of IFN-γ as compared to controls, this lymphokine was found not to be the main inhibitor of in vitro granulopoiesis.

Other, non-IFN related factors were potent inhibitors of both the mature and the immature precursors for GM-CFC, could act across the species barrier and were of low molecular weight. Also PBMC from healthy donors produced a non-IFN mediated GM-CFC inhibitory factor, but to a lesser degree and acting only on one type of myeloid precursors. The possible implications of these findings in relation to the etiology of AA will be discussed.



FUNCTIONAL EFFECTS OF CD2 AND CD3 ANTIBODIES

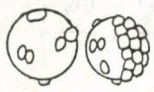
/Gy.Görög, A.Bátory and A.L.Vecchia/ Leucocyte Typing III. McMichael, ed. Oxford University Press, 1987.

The Workshop CD2 and CD3 sets were screened in a number of functional assays and the endpoint /minimal concentration of antibody required/ of some of the effects were determined. The enormous differences found may hopefully eventually be related to epitope specificity. We have shown here that proliferation and helper effect can be dissociated using either CD2 or CD3 antibodies. The CD2 pathway appears to provide an additional immune-response enhancing signal when helper cells have been activated by another mechanism.

THE EXPRESSION OF ACTIVATION MARKERS AND CD25 ANTIGEN ON PBL IN COMPARISON WITH IMMUNE FUNCTION AFTER ALLOIMMUNIZATION

/B.Kotlán, E.Gyódi, M.Benczúr, K.Takács, T.Szabó, J.Troppmair, K.Onody, I.Petri, G.Kaiser, M.Kassai, Ch.Huber and G.Gy.Petrányi/
Third International Workshop and Conference on Human Leucocyte Differentiation Antigens, Oxford, 21-26 Sept. 1986.

Activation markers are mainly characterized on the basis that they are expressed only on cells in proliferation or differentiation states, induced by either mitogens or antigens. Therefore, selection of monoclonal antibodies detecting activation antigens is usually performed on cells obtained from mitogen-stimulated cultures, from mixed lymphocyte cultures, or on established cell lines /originating from various leukaemias or after viral induction/. In our Workshop studies we posed the question whether activation antigens could be detected in vivo after immune stimuli in a similar manner to the in vitro studies. We have found, that, after alloimmunization with non-histocompatible leucocytes, the activation of the immune system is manifested by an increased expression of class II histocompatibility antigens and IL-2 receptors in parallel with the expression of other activation markers on PBL. However, big differences were found with regard to the number of cells expressing particular activation antigens before and after alloimmunization.



NEWCASTLE DISEASE VACCINE /LA SOTA/ STRAIN SPECIFIC MONOCLONAL ANTIBODY

/J.Erdei, J.Erdei, K.Bachir, E.F.Kaleta, K.F.Shortridge and B.Lomniczi/
Archives of Virology, 1987. Vol.96. p.265-269.

Newcastle disease virus vaccine strain /La Sota/ specific monoclonal antibody /La-1/ was produced by immunizing mice with isolated glycoproteins of strain La Sota. This antibody was recognized only in the ELISA test in which it bound exclusively to La Sota strain out of a range of over 300 lentogenic, mesogenic and velogenic strains examined.

MONOCLONAL ANTIBODIES TO ADENOVIRUS TYPE 35 HEXON

/É.Ádám, J.Erdei, A.Lengyel, Gy.Fejér, J.Fachet and I.Nász/
Intervirology, 1987. Vol.27. p.9-16.

A panel of 37 monoclonal antibodies /MAbs/ directed against adenovirus type 35 /AV35/ hexon was studied by indirect enzyme-linked immunosorbent assay /ELISA/ and passive hemagglutination /HA/ methods. Nine heterologous hexon types and the homologous type were used to determine the reactivity pattern /RP/ of the MAbs and to study the antigenic relationship among the different hexon types. Eleven types of RPs were shown using ELISA and seven types were shown using the HA test. In the case of six MAbs, the RPs were identical in both assay systems; 31 MAbs showed some differences when the resulty of the two methods were compared. The common epitopes of the dif-

ferent hexon types studied seem to be characterized as genus, subgenus, intersubgenus, and intertype specificities. The type-specific determinant of AV35 hexon could be detected by several MAbs. The antigenic relationship seems to be closer between the two oncogenic subgenera /A and B/ of adenoviruses, whereas the antigenic relationship to AV35 hexon is somewhat looser for subgenera D, and E. Hexon types of subgenus C showed the greatest differences in antigenic structure compared with the AV35 hexon.

THE FIGHT AGAINST PLASMIDS

/J.Molnár/
Tudomány, 1987. No.3. p.32-33.

Plasmids are DNA molecules responsible for the inheritance of the resistance against antibiotics, of the metabolic processes, and of other properties which is completely independent of the chromosomes of their host bacteria. The resistance against antibiotics carried and transferred by the plasmid makes it possible for the bacteria to grow in the presence of antibiotics. Since this created a great problem in the therapy of infectious diseases, not only new antibiotics are needed but a new strategy should also be developed to eliminate the plasmids restoring the effectivity of the known pharmaceuticals again. The starting point of a new approach in this fight is based on the new invention of the authors /discovered by chance/ that some membrane-active psychopharmacons, like chlorpromazine and pipolphen, decreased the resistance of E. coli against tetracyline, chloramphenicol, streptomycine, and sulfonamides. It was shown that these drugs removed the plasmids from the host-bacteria and inhibited the propagation of resistance. The main feature of the known plasmid-deletors was their intercalation into the plasmamembrane of the bacteria. The above-mentioned tricyclic psychopharmacons act via a different mechanism. It is assumed that they inhibit all the three basic mechanisms of the existence of the plasmid: amplification, partition, and conjugative transfer.

The experimental experiences, the computer-aided drug-planning, and the quantumtechnical estimations are promising for finding a new technique for the plasmid deletion.

ANTIMETABOLITES, ANTITEMPLATES, DUAL ANTAGONISTS: THE SELECTIVE INHIBITION OF DNA SYNTHESIS IN THE CHEMOTHERAPY OF CANCER

/T.Bárdos/
Kémiai Közlemények, 1987. Vol.64. No. 1-2. p.1-28,

Several new approaches of the chemotherapy of cancer are reviewed based on the author's forty years long research work. Among the antimetabolites, the nucleosides and pteridines are discussed and the importance of the combinative dosage is emphasized. The concept of dual antagonists was developed to enhance the effect and to promote the application of the combined therapy. In the dual antagonists, the structural elements of two or more synergetic chemotherapeutics are incorporated into one molecule, thus they have many advantages, e.g. synchronizing effect, greater ac-

tivity, etc. A good example for dual antagonists is the aziridine family which is characterized by low toxicity, sensitizing affect to X-ray irradiation, and cholinesterase inhibition. Another new approach of chemotherapy is the production of antitemplates, i.e. high-molecular structural analogues of DNA molecules.

THE EFFECT OF VARYING ILLUMINATION ON IMPRINTING OF TETRAHYMENA BY INSULIN

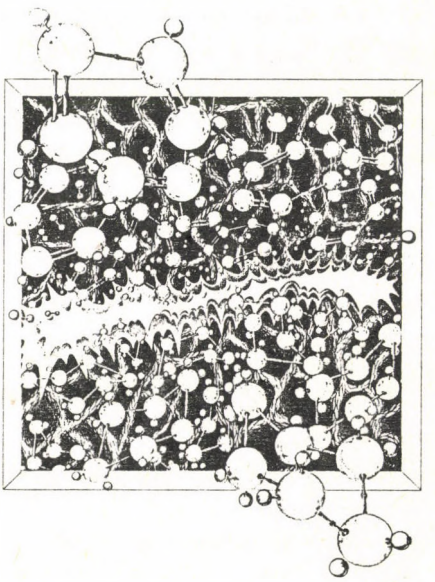
/L.Kőhidai, Zs.Darvas and G.Csaba/
Acta Microbiologica Hungarica, 1987. Vol.34. No.3-4.

Tetrahymena pyriformis cultures were imprinted with insulin. Hormone binding was reduced in the dark, but alteration of dark and light periods were in this respect more effective than the dark itself. The deviations observed may be attributed. besides the reduced insulin binding by the imprinted cells in the dark, to an enhanced bidding by the non-imprinted control cells kept in the dark. It is suggested that the dark-induced structural transformation of the membrane, manifesting among others in a changed hormone binding, may be caused by alterations in haem synthesis due to varying illumination.

THE EFFECTS OF CANNABINOIDS AND CANNABISPIRO COMPOUNDS ON ESCHERICHIA COLI ADHESION TO TISSUE CULTURE CELLS AND ON LEUKOCYTE FUNCTIONS IN VITRO

/J.Molnár, I.Petri, I.Berek, Y.Shoyama and I.Nishioka/
Acta Microbiologica Hungarica, 1987. Vol.34. No.3-4. p.233-240

Δ^9-Tetrahydrocannabinol, cannabidiol, cannabidiolic acid, tetrahydrocannabidiolic acid, cannabispirol, cannabispirone, and cannabispirenone in a low concentration did not affect the adhesion of Escherichia coli on cultured HEp-2 cells. Cannabinoids at 10^{-6} M increased the chemiluminescence of human polymorphonuclear leukocytes, while the cannabispiro compounds failed to enhance the oxidative burst of leukocytes. In lymphocyte and granulocyte function tests /E- and EA-rosette formation, blast transformation of T-lymphocytes in the presence of phytohaemagglutinin and concanavalin-A, ADCC and phagocytosis/ all compounds displayed immunosuppressive effect at 1.5×10^{-5} M. Tetrahydrocannabidiolic acid exerted the weakest immunosuppression on human leukocyte functions.



BIOENGINEERING
ANALYSIS APPARATES

DETERMINATION OF VALIN, LEUCIN, I-LEUCIN, METIONIN AND PHENYL-ALANIN IN FERMENTATION BROTH BY QUADOPULE MASS SPECTRO-METER

/Gy. Sántha, J. Szilágyi/
Proceedings of the 4th European Congress on Biotechnology, 1987. Vol.3. p.110-113.

A rapid and specific method was developed for simultaneous measuring of five amino acids in fermentation broth by a Q 300 C type Quadrupole Mass Spectrometer.

The basis of the method is the reaction of ninhydrin with free amino acids that is widely used in chromatography for enhancing the sensitivity of detection.

MASS SPECTROMETRIC METHOD FOR DETERMINATION ABSOLUTE CONCENTRATION OF PHYSICALLY DISSOLVED CARBON DIOXIDE IN THE FERMENTATION BROTH

/J. Szilágyi/
Proceedings of the 4th European Congress on Biotechnology, 1987. Vol.3. p.85-88.

A mass spectometric method was developed for determination the absolute physically dissolved CO_2 content of fermentation broth. During a penicillin fermentation, carrying out in the pilot plant of BIOGAL Pharmaceutical Works, the partial pressure of physically dissolved CO_2 was monitored continuously by QMS. Sample was taken by a special designed ceramic filter sampling system. The CO_2 content of filtrated broth was measured by QMS from untreated and treated sample respectively. Calculated data was used for calibration physically dissolved CO_2 content.

FERMENTATION TECHNOLOGIES

/S. Csiky/
Természet Világa, 1987. Vol.118, p.179-181

Applied biological processes are mainly related to the fermentation. In Hungary, there is a great tradition of the fermentation technology. The optimization of the biotechnological process is based on the appropriate choice of the bioreactor and the environmental parameters. There are three main types of fermentor configurations: conventional reactors with mechanical stirring, tower- or tube-reactors, and loop-reactors. The latter has three subtypes depending on the mood of circulation of the fluid: air-lift, propeller, and JET loop-reactors including the HTP-JET circulation system based on a Hun-

garian patent and applied at the Technical University of Budapest and at G.Richter Pharmaceutical Co. Microorganisms can be cultured in submerse cultures by means of continuous or batch fermentation technology. In Hungary, the basic batch technology is generally applied, while the fed-batch technology is under development. The most economical technique is the repeated fed-batch technology which needs a sophisticated technical level. The Hungarian pharmaceutical industry /Biogal, G.Richter Co./ applies the most up-to-date analytical methods following the process. Among these, the quadrupole mass spectometry - microprocessor system should be mentioned developed firstly in Hungary be the ATOMKI Institute. The QMS system has been introduced in the penicylline production at Biogal. Combines systems autoanalyzer- and HPLC-fermentor are also used as on-line analyzers.

ANOTHER STEP IN CELL BIOLOGY: APPLICATION OF THE FLOW CYTOMETRY

/S.Damjanovich and R.Gáspár/
Magyar Tudomány, 1987. No.3, p. 173-179

Flow cytometry is a high-speed optical analysis and electrohydrodynamic separation of cells or biological particles based on light-scattering or fluorescency. Many thousands of cells can be studied within a second and the results can be stored and recalled. Flow cytometry is widely applied at different fields nowadays based on the development of new selective staining techniques. It is possible, therefore, to follow the effect of a drug on the cell by monitoring the changes in the membrane potential due to the action of the drug. Another field of application is the measurement of the distance between elements of the cell surface /or intracellulary localised/ by the authors' method named fluorescent resonance energy transfer /FRET/ analysis. The technique is based on the application of two fluorescent dyes; the energy primary excitation of the first one excites the fluorescence of the other. Using this method, the dynamic changes could be determined within the membrane /i.e. receptor-receptor interactions/. Flow cytometry is also used in animal breeding for the selection and determination of viability of sperms. In the plant physiology, protoplasts can be separated and studied. This technique will likely achieve some other applications. It is already used in immunology and in the medical diagnosis of surface antigens in malignant diseases.

FLOW CYTOMETRIC DETERMINATION OF THE SPERM CELL NUMBER IN DILUTED BULL SEMEN SAMPLES BY DNA STAINING METHOD

/T.Takács, J.Szöllősi, M.Balázs, R. Gáspár, L.Mátyus, G.Szabó, L.Trón, I.Resli and S.Damjanovich/
Acta Biochimica et Biophysica Hungarica, 1987. Vol. 21. No.1.

Flow cytometric determination of the number of bull sperm cells showed that the number of spermatozoa measured by light scattering may considerably differ from the actual number of sperm cells in the

samples, depending on the proportion of contaminating particles, similar in size to sperm cells. No accurate information can be obtained from the sum of live and dead cells distinguished by means of double fluorescence staining, since a part of the sperm cell population is in a transitory state i.e. between the viable and dead states, so it cannot be stained by either dye.

The number of spermatozoa in the sample can be determined very accurately if the sample is treated first with Nonidet-P-40 detergent then stained with propidium iodide. With this procedure the DNA content of each cell nucleus can be labeled and, through detecting the fluorescence signals, the actual sperm cell count of the sample can be determined with the accuracy of 95-98 %.

FLOW-CYTOMETRIC EVALUATION OF BULL SEMEN. III. BIO-PHYSICAL ANALYSIS OF DOMESTIC AND IMPORTED DEEP-FROZEN SEMEN SAMPLES

/T.Takács, J.Szöllősi, M.Balázs, R. Gáspár Jr., L.Mátyus, G.Szabó Jr., L.Trón, I.Resli and S.Damjanovich/ Magyar Állatorvosok Lapja, 1987. Vol.42. No.1. p. 52-55.

Comparative analyses of 9 imported and 12 domestic deep-frozen samples/originating from 3 bulls each of 4 stations/ were carried out by conventional laboratory methods /cell-counting by haemocytometre, microscopic examination of motile cells/ and by modern cytometric procedures. Comparing the two methods, significant differences were found between the results of the two examination procedures, concerning the cell counts/ dose and the proportion of living sperm cells. Reproducibility of flowcytometric measures /CV=2 to 5 %/ were significantly better than that of laboratory measures /CV=25 %/. On the basis of the results of flow-cytometric measures, it was pointed out that the total sperm counts of inseminating doses of 9 imported and 12 domestic semen samples were comparable. However, the average proportion of living sperm cells was significantly higher /$p<0.05$/ in the domestic samples than in the imported ones.

FLOW-CYTOMETRIC EVALUATION OF BULL SEMEN. IV. FOLLOW-UP OF CHANGES IN THE QUALITY OF BULL SEMEN PLOTTED AGAINST THE TIME OF EQUILIBRATION BEFORE DEEP-FREEZING

/T.Takács, J.Szöllősi, M.Balázs, R. Gáspár Jr., L.Mátyus, G.Szabó Jr., L.Trón, I.Resli and S.Damjanovich/ Magyar Állatorvosok Lapja, 1987. Vol.42. No.6. p.347-353.

One ejaculate each of three bulls was divided in two parts. They were equilibrated with TRIS and Jondet diluents at +4°C. Deep-freezing of samples in 0,25 ml plastic straws was carried out with half an hour intervals to compare the diluents, to determine the optimal time of equilibration and the grade of cellular destruction during processing. The investigation was performed by flow-cell-fluorometer. Simultaneously, conventional laboratory methods were also used.

It was found that destruction of sperm cells during equilibration was higher by 9 % in Jondet diluent than in TRIS diluent. The losses during freezing were comparable, thus the number of living cells was higher by 8,3 % in ejaculates processed in TRIS diluent. The optimal time of equilibration varied between 3 and 18 hours in the case of Jondet diluent, showing a great individual variance. The same for TRIS diluent was 26 hours, however satisfactory deep-freezing results were also obtained in case of two bulls between the 10th and 16th hours.

HEAT TRANSFER COEFFICIENT FOR VERTICAL TUBE BAFFLES IN MIXED REACTOR

/G.Havas, A.Deák and J.Sawinsky/
Magyar Kémikusok Lapja, 1987. Vol.42. No.12. p. 457-460.

Heat transfer coefficients for vertical tube baffles have been investigated in model vessels of different dimensions. A modified Reynolds number has been introduced into the heat transfer equation. This equation has been in good agreement with experimental data measured in a fermenter of 100 m^3 volume.

CLUSTER ANALYSIS OF CARBON SOURCE UTILIZATION PATTERNS OF TRICHODERMA ISOLATES

/L.Manczinger and G.Polner/
Systematic and Applied Microbiology, 1987. Vol.9. p.214-217.

The ability of 65 Trichoderma isolates to grow on 127 carbon sources was tested. Cluster analysis was performed on the basis of the data derived from the utilization of the 42 carbon sources which gave reproducible results. Partial correlation was found between the carbon source utilization patterns and previously designated species aggregates.

Distinct groups within the same species aggregates consisted sometimes of strains derived from very distant geographical areas. Four groups of Trichoderma harzianum and three groups of Trichoderma hamatum could be clearly identified.

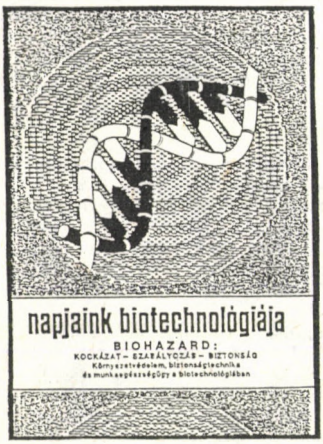
Carbon source utilization appears to provide a reliable method for the identification of species within the species aggregates of the problematical genus Trichoderma.

POTENTIAL OF ORGANIC SOLVENTS IN CULTIVATING MICRO-ORGANISM ON TOXIC WATER-INSOLUBLE COMPOUNDS

/J.M.Rezessy-Szabó, G.N.M.Huijberts and J.A.M.de Bont/
Proceedings of an International Symposium held at Wageningen, The Netherlands, 7-10 December 1986.

The effects of seven organic solvents on nine different aerobic bacteria were tested. The reaction of growing cells and of immobilized cells to the organic solvents was similar. Solvents with a relatively high logP-value were not harmful to cells but it was also noticed that great differences exist amongst the various bacteria tested in their reaction to the solvents. Using a benzene-utilizing bacterium it

was observed that dibutyl phtalate is a suitable solvent in circumventing benzene toxicity during growth of the organism. From the results it is expected that organic solvents will be of use in cultivating micro-organisms on other and more toxic water-insoluble compounds.



USE OF CHROMATOFOCUSING FOR SEPARATION OF β-LACTAMASES

VIII. ANALYTICAL CHROMATOFOCUSING OF CHROMOSOMAL CEPHALOSPORINASES FROM FOUR KLEBSIELLA STRAINS

/S.Gál, A.Tar, M.Frommer-Filep, B. L. Toth-Martinez and F.J.Hernádi/
Journal of Chromatography, 1987. Vol. 403. p.217-224.

Although still there are Klebsiella strains which do not harbour plasmids and produce constitutive chromosomal β-lactamases, recently clinical isolates were found in ever increasing numbers carrying mainly TEM-, CARB- and OXA type R-factors. We selected four chromosomal cephalosporinase producing Klebsiella strains to study the pI values of the enzymes and their simultaneous separability from accompanying proteins by chromatofocusing techniques. We compared pI values of the pure and the crude preparations: K. pneumoniae L1 SC 10436: pI_{pure}= 6.4, pI_{crude}= 6.42; K. aerogenes K1 1082 E: pI_{pure}= 6.5, pI_{crude}= 6.5; K. oxytoca 1082 E: pI_{pure}= 6.42, pI_{crude}= 6.4; K. oxytoca 20: pI_{pure}= 7.62, pI_{crude}= 7.6. Excellent agreement of the pI values among each other, but occasional differences with those obtained by analytical isoelectrofocusing are attributed to methodological diversities and to the presence of satellite enzymes, known to exist in Klebsiella.

CORRELATION OF LIQUID DISPERSION AND OXYGEN TRANSFER IN BUBBLE COLUMN

/J.Holló, P.Miháltz, L.Czakó and J.Tóth/
Appl. Microbiol. Biotechnol. 1987. Vol.27. No.3. p.260-264.

In steady state, attained by continuous aeration after oxygen saturation of water in a bubble column, vertical composition distribution of liquid and gas phases has been determined. It has been assumed that, as a result of absorption in the upper section and vertical dispersion of dissolved oxygen flux, a closed oxygen circulation is created. Determination of the axial dispersion coefficient from hydrodynamic and oxygen transfer data verifies the mathematical model proposed. The results allow conclusions to be drawn about supersaturation and desorption and other phenomena expected in biological systems.

BIOTECHNOLOGY TODAY issues published in 1987

No. 1.
Plant Biotechnology, 2nd Symposium on Plant Cell Genetics and Tissue Culture
The publication presents the lectures of the 2nd Symposium on Plant Cell Genetics and Tissue Culture which was held in Budapest on 24th of April, 1986 in the following themes: molecular biology, cell genetics, morphologenesis, regulation, selection, reproduction.

No. 2.
Development tendencies of biotechnological industry and possibilities of development in our country (Study)
The study investigates the present state, importance and development of the new biotechnology in the world and in Hungary, the situation of crude materials, the products of biotechnology and the possibility for their practical application. Demonstrates the processes of technology and the assesment of equipments. Deals with the main targets in this area of this country.

No. 3.
Patenting in biotechnology (Review)
The review deals with the problems of legal aspects of biotechnology in the international special literature: Treaties regulating the trade of biological materials, Consultation and intellectual property in the practice of the American firms, Intellectual property and patenting in biotechnology, Criteria for patenting – novelty, Judicial problems of commercialization of biotechnology. The second part of the publication deals with the question of patenting in biotechnology of Hungary: Patent law and regulation for Hungarian biotechnology, Judicial practice of the National Invention Agency of Hungary in biotechnology, Problems in the unity of inventions and in the width of patenting at the genetic modification of microorganism.

No. 4.
Stockbreeding biotechnology 2nd Round-table conference
This conference was organized at Szerencs on 7– 8 October, 1986. The publication presents the lectures of the conference, illustrating the situation of stockbreeding in Hungary.

No. 5.
The prospective economical effect of plant biotechnology (Study)
The review of plant biotechnology in the world and in Hungary. Situation of R+D in the new biotechnology, aspects of the practical application, the development and the effect on the economy. The present state of manufacturing laboratories and equipments in Hungary.

No. 6.
Present state and prospects of development of animal breeding and veterinary science biotechnology (Study)
Biotechnology in animal breeding, biotechnological possibilities in animal feeding. Preparation of diagnostics and vaccines for animals.

No. 7.
Relation between the biotechnological research and development and the production.
IInd Technical Development Conference on Biotechnology,
Szeged, 27– 28th May, 1986.
The program and the lectures of the Conference.

No. 8.
Biohazard: Risk – Regulation – Safety.
Environmental and safety aspects, and occupational health in biotechnology. (Review)
Regulation policy in the USA and in Europe. Environmental risk in biotechnology. Risk assessment and questions of emission. Safety in biotechnology. Documents related with the regulation.

No. 9.
Ungarisch– Österreiches Symposium über Biotechnologie.
10. November– 13. November, 1986.
The lectures of the conference are presented in German and English languages.

No. 10.
III. Roundtable Conference on the Animal Breeding Biotechnology.
Hőgyész, 6– 7 October, 1987.
The lectures of the conference are presented.

No. 11.
VIII. Colloquium on Fermentation.
Balatonszemes, October, 1987.
The publication presents the lectures of the colloquium.

No. 12– 13.
Micromanipulation of bovine embryos and possibilities of the applications of this technology in animal breeding.
This work is based on the author's experiences in production of bovine monozygote twins and chimeras by micromanipulation - microchirurgy. The main factors influencing the effectivity are examined. Development of a model for the practical application and research.

FOOD INDUSTRY

EFFECTS OF HEATING ON BIOLOGICAL VALUE OF SOYABEAN

/J.Petres, Zs.Márkus, B.Czukor, É.Gelencsér and J.Gyebróczki/
International Biochemical Symposium on Food Proteins, 6-10 October, 1987 Balatonszemes, Hungary.

Soyabean, because of its high protein content and relatively well-balanced amino acid pattern, a potentially valuable protein source both for humans and animals. To increase the nutritional value of raw soyabean some antinutritional factors, mainly trypsin inhibitors must be inactivated. Different heat treatments are known to eliminate the adverse effects of antinutritional factors and produce soyabean products with good protein nutritional quality.
The aim of this study was to investigate the effect of dielectric heating on protein biological value in soyabean.

Whole soyabean /Glycine mex, var.: Ewans/ was heated at three different temperatures by a pilot plant dielectric equipment. Protein nutritional values of raw and treated samples were estimated by rat assay and characterized by Net Protein Ratio /NPR/, Net Protein Utilization /NPU/, True Digestibility /TD/ and Biological value /BV/. Trypsin inhibitor activity of the samples were measured.

It was found that the in vivo nutritional values of treated samples were significantly higher /NPR = 23.3-25.3, NPU = 58.4-61.9, BV = 65.7-77.8/ than the respective values of raw soyabean /NPR= 14.9-17.1, NPU = 42.1-42.5, BV = 52,7-54.9/. Remaining trypsin inhibitor activities of treated samples were 5-10 % of origin.

COMPARISON OF CELLULOLYTIC ENZYME COMPLEXES OF DIFFERENT FUNGAL ORIGIN

/M-Szakács-Dobozi, A.Halász, L. Vámos-Vigyázó/
British Polymer Journal, 1987. Vol.19. p.83-90.

The cellulolytic culture filtrates of Trichoderma reesei QM 9414, Aspergillus terreus OKI 16/5 and Penicillium verruculosum WA 30 were purified and separated into components by one-step preparative isoelectric focusing /IF/. The culture filtrates, the mycelia and the separated components were investigated for cellulolytic /filter-paper degrading /FPA/, carboxymethylcellulose degrading /C_x/ cotton hydrolyzing /C_1/, cello-

biase/ and proteinase B activities. The molecular weights of the cellulolytic fractions were determined by SDS-electrophoresis.

The culture filtrates differed in the proportions of the various cellulolytic activities. The analytical IF separation of the enzyme complexes resulted in a total of 28-31 protein fractions, mostly glycoproteins. The complexes of the different culture filtrates were separated, by preparative IF, into 28 fractions each. The greatest part of the activities could be recovered in fractions 8-18.

The activities in the fractions were recovered to different extents. The main protein components in the enzyme complexes were found to be endoglucaneses /C_x/ with pI values between 3 and 6.

Most of the endoglucaneses proved to be glycoproteins. FPA and cellobiase activities were recovered to various extents in the fractions of the three culture filtrates. Only small parts of the C_1 activities were recovered in the Trichoderma and Penicillium fractions. The originally low C_1 activity of the Aspergillus culture filtrate was recovered to 76 % in the separated fractions. Most of the C_x activities were found to be lost. However, the separation losses were found to be reversible: on combining the fractions, the activities originally present in the culture filtrate were nearly entirely restored.

The molecular weights of the fractions of the different culture filtrates covered the range 10 000 to 70 000 D. The IF fractions of similar activity patterns also showed similar molecular weight distributions.

The relatively large number of isoenzymes is assumed to be the result of the endocellular proteinase activity present, which splits off the cellulase enzyme components from proenzymes of high molecular weight during the fermentation process.

RADIOACTIVE CONTAMINATION OF AGRICULTURAL AND FOOD PRODUCTS

/P.A.Biacs/

7th World Congress of Food Science and Technology, Raffles City, Singapore 28 Sept to 2 Oct 1987.

Radioactive contamination of agricultural products and processed foods increases after control failures and accidents of nuclear power stations. The way of airborne radioactive particles and gases are traced by trajectorial estimations of meteorological surways. Solid particles /aerosols/ escaping from the reactor fall out in the environment accelerated by the effect of precipitation. Activity levels are measured by air collectors /Bq/m^3/ or sedimentation equipment /Bq/m^2, day/. Radioactive contamination can be accumulated on the surface of vegetables, fruits, grass, herbaceous plants, etc. Secondary environmental effects sometime enhance radioactivity level in an area causing epidemy. Radio-activity of soil shows remarkable differences in certain regions or even within a country like in the Eastern European and Scandinavian countries after the Chernobyl accident.

Elimination of radioactivity from the surface of agricultural and forestry products can be achieved by intensive rinsing, peeling or by the removal of heavily contaminated tissues or organs. Food processing is

facing a new risk by handling contaminated foodstuffs being over the limit of acceptance of a given country. Food exporting and importing countries react very diversely declaring their own limits of acceptance and requiring certificates from trading companies.

STUDIES ON THE CAROTENOID PIGMENTS OF PAPRIKA /CAPSICUM ANNUUM L. var Sz-20/

/P.A.Biacs, H.G.Daood, Cs.A.Pavisa and F.Gy.Hajdu/

Scientific International Technical-Development Symposium on Hungarian Paprika /Red Pepper/, Kalocsa-Szeged 1987. Sept.17-19.

The carotenoid pigments of paprika fruit were separated and identified by high-performance liquid chromatography /HPLC/ on Chromsil C_{18} reversed-phase column with 39:57:4 /v/v/v/ acetonitrile-isopropanol-water as the mobile phase under isocratic conditions and without prior saponificationof the samples.
Fatty acid carotenoid esters and unesterified hypophasic and epiphasic carotenoids were identified by thin-layer chromatographic methods /TLC/ as well as by the direct spectral scanning of the peaks of each component separated by HPLC.
Monoesters of capsanthin were found to contain mostly unsaturated fatty acids /$C_{18:2}$/ while diesters of both capsanthin and capsorubin contained saturated fatty acids such as C_{12}, C_{14} and C_{16}. The carotenoid esters were more stable, towards lipoxygenase /LOX/-catalyzed linoleic acid oxidation, than free pigment. Furthermore, capsanthin esters containing saturated fatty acids resisted the enzymatic oxidation better than the others did.
The changes taking place in the concentration of the individual coloured substances during ripening stages and processing of paprika were also studied with special attention to the red pigments.

HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHIC CONTROL OF THE STANDARD SPECIFICATION OF GROUND PAPRIKA

/A.Cs.Pavisa, F.Hajdu, Á.Hoschke, J.Bodnár and P.Biacs/

Acta Alimentaria 1987. Vol.16. No.2. p. 129-142.

Classification of commercially marketed ground paprika is based world-wide on the measurement of the overall pigment content as established by spectrophotometry subsequent to organic solvent extraction.
The measurable carotenoid content of ground paprika depends largely on the extracting solvent applied and the wavelength used for determination, thus, to avoid marketing problems it seemed expedient to study the conditions of determination as applied in the different countries /solvent used for extraction, wavelength applied, time of extraction/. Investigations were carried out by high-performance liquid chromatography /HPLC/ and spectrophotometry. Based on the results the use of a three-component solvent /e.g. chloroform-acetone-isopropanol, 2:1:1/ is suggested for extraction instead of the pure apolar sol-

vents generally applied. Determination is suggested to be made in the 500 to 510 nm wavelength region. The minimum extraction time was found to be 1 h.

SEPARATION AND IDENTIFICATION OF TOMATO FRUIT PIGMENTS BY TLC AND HPLC

/H.G.Daood, P.A.Biacs, Á.Hoschke, M.Harkay-Vinkler and F.Hajdu/
Acta Alimentaria, 1987. Vol.16. No.4. p.339-350.

High-performance liquid chromatography /HPLC/ and thin-layer chromatography /TLC/ techniques were applied for separation of the pigments of tomato fruit. More than 10 pigments were separated and identified in ripe fruit of ventura cultivar of the tomato plant.
A new mobile phase for both TLC and HPLC system was developed and better separation for the major as well as the minor pigments was achieved. These procedures were found to be reproducible, rapid and not to cause remarkable damage to the separated pigments or to the adsorbents of the chromatographic systems.
The individual pigments of tomato fruit were identified according to their physico-chemical and chromatographic properties /retention time in HPLC, R_f-value in TLC and visible-absorption spectra/.
The fresh ripe fruit accumulated mostly lycopene, neurosporin, β-carotens and lutein, besides neoxanthin, xanthophylls, chlorophylls and prolycopene as minor pigments.
The new mobile phase of HPLC was also applied to the separation of red pepper pigments as a comparison sample.

SEPARATION AND IDENTIFICATION OF CAROTENOID-ESTERS IN RED PEPPER /CAPSICUM ANNUUM/ DURING RIPENING

/P.A.Biacs, J.Bodnár, Á.Hoschke, A.Cs.Pavisa, H.Daood, F.Hajdu and N.Kiss-Kutz/
The Metabolism, Structure, and Function of Plant Lipids, 1987. Plenum Press New York and London

Natural pigments of red pepper are mostly bound to fatty acids as carotenoid esters. In this paper the composition changes of red pepper lipids, carotenoids, mono- and disaccharides during ripening are discussed. The formation and the decomposition of more than 20 components were followed.

CHARACTERIZATION OF TOMATO LIPOXYGENASE

/P.A.Biacs and H.Daood/
The Metabolism, Structure, and Function of Plant Lipids, 1987. Plenum Press New York and London

Lipoxygenase enzyme is responsible for the oxidation of fatty acids and their glycerides containing methylene interrupted system of double bonds such as linoleic, linolenic and arachidonic acids. The presence of lipoxygenase in the economically most important tomato variety in Hungary /ventura/ has been proved. In this paper the extraction, the purification steps and the characterization of the enzyme are described.

PLANT CULTIVATION

OXYGEN TRANSFER AND RHEOLOGICAL CONDITIONS AND THE EXPERIENCES ON SCALE EXTENSION OF THE FERMENTATION PROCEDURE OF GENTAMYCIN

/I.Varga/ Doctor's Theses

The experimental work aimed at studying the oxygen transfer conditions in pilot plant-scale and production-scale fermentation of gentamycin in order to augment the batch size by installation of fermentors of 150/100 m^3.

For this purpose, a comprehensive study was conducted upon the environmental effects on the process of fermentation, more exactly, on the volume transfer coefficient of oxygen. Rheology of the ferment liquor was investigated and the non-Newtonean apparent viscosity was related to $k_L a$. As an important factor of scale extension, the peripheral speed of the stirrer had to be maintained at a constant level when changing for different fermentor sizes, such as from 0.3/0.2 to 150/100 m^3 by adjusting the speed of rotation, the air flow rate, and the dilution in order to balance the desired concentration of oxygen dissolved. By maintaining the peripheral speed of the stirrer, the shear rate did not exceed the critical level. In the large-scale production, $k_L a$ of the ferment liquor corresponded to that in the pilot plant.

EFFECT OF UPSTREAM SEQUENCES ON THE STRENGTH OF AN RNA PROMOTER IN THE RIBOSOME OF ESCHERICHIA COLI

/Á.Pethő/ Doctor's Theses, 1986. Presented partly as a poster at the 17th FEBS Meeting, Berlin-West, 1986: Á.Pethő, J.Belter, I.Boros, P. Venetianer: Contribution of Upstream Sequences to the Strength of a Promoter in E.Coli.

P_1 promoter of the deletion rrnB gene cloned in the plasmide vector was subjected to deletion analysis. A series of overlapping deletions was produced in the upstream AT-rich region by in vitro exonuclease digestion with BAL31. It was established that, under some nearly physiological conditions, the lower an in vitro transcription activity the deletion promoters had, the longer the missing fragment was in the AT-rich region. It was supposed that this involved region would also contribute to the enhanced strength of the promoter.

CLONING OF RNA GENE IN THE RIBOSOME OF CEPHALOSPORIUM ACREMONIUM AND PREPARATION OF VECTORS BY USING ITS FRAGMENTS

/G.Járai/ Doctor's Theses, 1986.

Techniques for isolation of C. acremonium DNA and ribosomal RNAs were developed. RNA gene fragments of the ribosome were cloned in E.coli and in plasmids pBR322 and pNEO and a 3.2-kb EcoRI, a 2.8-kb BglII, and an 8.0-kb BglII fragments were isolated. It was demonstrated by restriction mapping, e.g. nuclease mapping, and by a partial sequence analysis that the complete ribosomal RNA gene was represented in the cloned fragments. The gene fragments of various functions and the aminoglycoside-3'-phosphotransferase gene were incorporated into the plasmides of E. coli, producing vectors that were replicated and selected in E.coli and most likely in C. acremonium as well.

STRUCTURE OF RIBOSOMAL RNA GENES

/G.Kelemen/ Doctor's Theses

Sequence analysis of the 1130 bp long fragment of the non-transcripting upstream region of RNA gene in rat ribosome was performed and the possible functions of these sequences were discussed. An rDNA fragment of an 8.0 kb long Novikoff hepatome was cloned and the obtained clone was characterized. Instability of rDNA clones was also studied. The site of deletion and the nucleotide sequence of its surrounding were determined in the deletion plasmides produced. The possible mechanism of deletion of sequences between the direct repetitions was discussed.

CHLORAMPHENICOL ACETYL TRANFERASE ACTIVITY IN BRASSICA spp.

/E.Balázs and J.M.Bonneville/
Plant Science, 1987. Vol.50. p.65-68.

Upon discovery that Brassica campestris leaf extracts harbour some chlorampenicol acetyl transferase /CAT/ activity, a systematic screening of plant tissue for this activity, so far only reported for prokaryotic microorganisms, has been conducted. Results were negative for three solanaceous plants as well as for the Cruciferae Arabidopsis thaliana and Orychropragmus violaceus. By contrast, the three tested species of the Cruciferae genus Brassica exhibit significant CAT activity. The Brassica CAT activity is much more heat labile than the enzyme encoded by the bacterial transposon, Tn9, that is commonly used as a reporter in gene fusion experiments.

APPLICATION OF IN VITRO TECHNIQUES IN CEREAL BREEDING
I. SOMACLONAL VARIATION

/F.Sági/
Növénytermelés, 1987. Vol.36. No.3. p.203-210.

Present tasks of cereal breeding are certainly substantial in both quantity and quality sense. In performing these tasks, biotechnological methods /production of somaclonal variants and androgenetic haploids, protoplast fusion, recombinant DNA technique/ play a more and more

important role. From the arsenal of biotechnology, for the time being the somaclonal variants and the androgenetic /microscope-derived/, rediploidized or spontaneously reduplicated haploids can be used directly in cereal breeding. Although somatic clonal variants can be produced quite easily and in a relatively short time from the staple cereals, the efficiency is still dependent on a number of largely unknown exogeneous and endogeneous factors, like nutrient medium, culture circumstances, genotype, physiological condition of the donors, kind of the explants etc. It is difficult to influence the number and nature of the variants. Moreover, the genetic and metabolic mechanisms regulating the redifferentation processes are not sufficiently clarified yet. It can be explained by these reasons, why only one cereal somatic variant /in rice/ became a variety up this date. However, other valuable somatic variants /e.g. Helminthosporium-resistant oats, amino acid overproducer maize/ have been already found, and their scale could be further enlarged via plant regeneration from callus cultures of various distant hybrids. Based upon the latest results, improvement of directed production of somaclonal variants and increase of their importance in cereal breeding can be experienced.

APPLICATION OF IN VITRO TECHNIQUES IN CEREAL BREEDING
II. HAPLOID-INDUCTION, GAMETOCLONAL VARIATION

/F. Sági/
Növénytermelés, 1987. Vol.36. No.5. p.385-394.

In the cereal breeding, besides induction of somaclonal variants, importance of the in vitro haploid techniques /anther and ovary culture, bulbosum-method/ seem to be steadily growing. Although these techniques are also unable to produce perfect varieties per se, using the dihaploids /DHs/ developed from the regenerants by colchicine treatment or spontaneous chromosome doubling, time of variety improvement can still be shortened, and simultaneously, its genetic basis can be widened. In spite of its dependence on various internal and external factors /e.g. genotype, physical condition of the donors, developmental stage of the microspores, pretreatment, culture temperature, nutrient medium/, as well as on the number of regenerated albino plants, the in vitro androgenesis furnished valuable plant material from which more wheat and rice varieties could already be developed. New barley cultivars have been also successfully produced by means of haploids from some barley X Hordeum bulbosum crosses. The androgenesis is capable to generate positive, homozygote genetic variants. Therefore, it might become a generally applied biotechnological method of cereal breeding, provided the efficiency of microscope embryogenesis and regeneration frequency of green, spontaneous DH plants could be increased.

IN VITRO CONSERVATION OF POTATO GERMPLASM IN HUNGARY

/L.E.Heszky and M.Nagy/
Biotechnology in Agriculture and Forestry 3. Potato
Springer Verlag, Berlin Heidelberg

New York London Paris Tokyo

The different techniques of in vitro conservation of potato reveal methods of overcoming the problems that existed in the late 1970s. The main features of these methods are:

1. Unlimited storage.
2. Unlimited propagation potential.
3. Truly virus-free stocks are available.
4. Low probability of genetic changes.
5. Variety-independent.
6. High survival rate.
7. No marked changes in productivity.
8. Relatively simple, reliable and safe.

With regards to Hungary, more than 100 old and new potato varieties are conserved in vitro in the Hungarian Gene Bank. Virus elimination, testing and micropropagation have been used in breeding programme. Recently, in vitro methods in seed-potato production have been initiated in Hungary.

PAST AND FUTURE OF THE RESEARCH OF RHYSOBIA IN HUNGARY

/K. Szende/
Növénytermesztés, 1987. Vol.36. No.2. p.125.

THE IMPORTANCE AND ROLE OF BIOTECHNOLOGY IN AGRICULTURE
Scientific Conference, Veszprém, 1986. VEAB, 1987.

Induction of Androgenetic Haploids in Wheat and Maize
B. Barnabás

Application of Bioengineering in Improvement of Potatoes
Mrs. L. Heszky

In vitro Deviration and Micro-Propagation of Fruit-Tree Species
J. Vértessy

Application of Plant Tissue Cultures to the Acceleration of Toxicological and Nutrient Supply Tests
M. László

In vitro Procedures in the Improvement of Fruits
I. Simon and J. Zatykó

Micro-Propagation of Ornamental Plants in Greenhouses
J. Retkes

Experiences from the Research into Bioengineering and Gene Manipulation in Breeding of Sheep
E. Gergátz

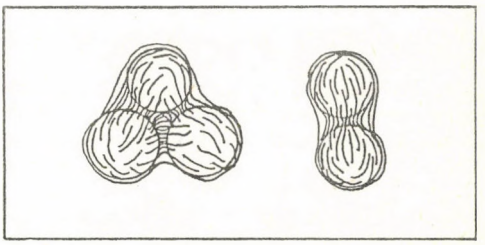
The Status and Current Results of the Research into the Genital Control of Cattles
J. Ivánc sics

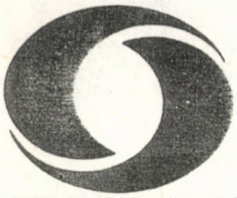
The Use of Cultured Lactic Acid Bacteria in Ensilation

a/ Investigations on Some Factors Influencing the Effectiveness of Starter Strains

b/ Effect of the Inoculation of Cultures of Lactic Acid Bacteria on the Process of Fermentation

F. Baintner, J. Schmidt, J. Szigeti, J. Varga.





STOCK BREEDING

RESULTS AND THEIR EVALUATION FROM THE RESEARCH INTO BIO-ENGINEERING IN THE ANIMAL BREEDING IN HUNGARY

/J.Becze/
Magyar Állatorvosok Lapja, 1987. Vol.42. /109/ No.8. P.495-498.

POSSIBILITIES TO THE APPLICATION OF BIOENGINEERING IN THE ANIMAL BREEDING

/T.Gere/
Állattenyésztés és Takarmányozás, 1987. Vol.36. No.2. p.115-124.

POSSIBILITIES TO THE PRACTICAL APPLICATION OF GROWTH HORMONES IN THE ANIMAL BREEDING

/F.Kutas/
Folia Biotechnologia, 1987. No.16. p.1-25.

THE 1st "OSZKÁR WELLMANN" SCIENTIFIC MEETING

Magyar Állatorvosok Lapja, 1987. Vol.42. p. 139-145.

Scientific meetings in memoriam Oszkár Wellmann, Professor of the Veterinary School of Budapest /1910-1943/ will be organized in every two years in the future. The first meeting was held at Hódmező-vásárhely, 28-29 October, 1986. It gave an overview on the scientific research of the Faculty of Veterinary Hygieny. The introductory lecture was delivered by Dr. Zoltán Csomós entitled: "The Economical Production of Animal Products". It was followed by more than 40 lectures or posters that summerized the recent scientific results of the Faculty.

THE SECOND ROUND-TABLE DISCUSSION ON THE BIOTECHNOLOGY OF ANIMAL BREEDING IN HUNGARY
Szerencs, 7-8th October, 1986.

Magyar Állatorvosok Lapja, 1987. March, p.186.

PRENATAL FATE OF PARTHENOGENETIC CELLS IN MOUSE AGGREGATION CHIMAERAS

/A.Nagy, A.Páldi, L.Dezső, L.Varga and A.Magyar/
Development, 1987. Vol.101. p.67-71.

Parthenogenetically activated BCF1 and fertilized BALB/c embryos were aggregated to form chimaeras. The fate of the parthenogenetic component was followed in the conceptus during the second half of gestation. The results indicate an early

strong selection against parthenogenetic cells in the extraembryonal part, which is presumably complete by term, and a weaker selective process in the embryo. During early development, parthenogenetic cells have nearly normal developmental potency in the embryo, which allows their balanced contribution in the chimaeras on day 12. Later, this contribution declines significantly resulting in an unbalanced relation to the advantage of the fertilized counterpart. From the results, we suggest that gametic imprinting may play a role not only in the key steps of preimplantation and early postimplantation, but later in cell and tissue differentiation.

QUALIFICATION OF RECIPIENTS AFTER TRANSFERRING DEEP-FROZEN AND FRESH EMBRYOS, BY MEASURING THE SERUM PROGESTERONE LEVELS

/S.Cseh, L.Molnár and L.Solti/
Magyar Állatorvosok Lapja, 1987. Vol. 42. No.6. p. 355-357.

For the qualification of recipients, besides oestrual data and rectal examination, changes of serum progesterone levels were also determined. Of 51 Hungarian red spotted, 1.5-2.5 years old recipient breeding heifers, 25 received fresh and 26 deep-frozen embryos. The cycle of animals was synchronized by the repeated administration of 30 mg of prostaglandin /Enzaprost-F inj. Chinoin/. Blood samples were collected three times from the heifers: at the time of second administration of prostaglandin, during heat and at the time of embryo transfer. The concentration of progesterone was determined by an enzymimmunoassay /EIA/ after petrolether extraction. The progesterone levels were evaluated subsequently, with the knowledge of the results of rectal pregnancy examination performed between the 70th and 90th days after embryo transfer. Of 51 heifers, 28 /55 %/ became pregnant. Changes of progesterone levels in pregnant and non-pregnant recipients between blood samplings are shown in the Table. No differences were found in the progesterone levels of recipients received fresh or deep-frozen embryos. Similarly, differences could not be detected in the progesterone levels of "non-pregnant" and "pregnant" groups measured at the time of synchronization and embryo transfer. However, significant differences /$p<0,1$/ were found in these two groups in the progesterone levels measured during the heat. The progesterone levels were lower in the pregnant heifers - both in recipients receiving fresh embryos and in those receiving deep-frozen ones - than in the non-pregnant heifers.

MONITORING THE EFFECTS OF GnRH ANALOGUES IN POST PARTUM DAIRY COWS

/T.Takács, Á.Balogh, A.Selmeczi/
European Society of Human Reproduction and Embriology, European Sterility Congress Organization, 27-30 Sept. 1987. Budapest.

One hundred and fifty cows were treated with different amounts of four newly synthetised superactive GnRH against ana-

logues compared with fixed doses of two marketed reference preparations /Receptal, HOECHST and Ovurelin, REANAL/ in the early post partum period /days 15 to 30 post calving/. Blood progesterone levels were assayed in samples taken every 3rd day, during a 24 day period from the day of treatment, using radioimmunoassay and the patterns were analysed as the index of ovarian activity.

Blood samples were also taken before one i.m. bolus injection of the analogues, and after 20, 40, 60, 120, 240 and 360 minutes post treatment to determine the pituitary activity /gonadotropin release/ by assaying bLH and bFSH concentrations using heterologous bLH and bFSH radioimmunoassay.

There was a significant increase in LH levels with a peak at the 120th minute in case of all examined new analogues independent of their doses /100, 250 and 500 μg respectively. The FSH profiles did not show such regularity although marked increase of their serum levels was observed, too. The increase of FSH levels after treatment was much more significant in case of one of the new analogues compared to the others. The ovarian activity was more regular in groups treated with GnRH analogues compared to the others. The ovarian activity was more regular in groups treated with GnRH analogues than in the control group. However at the stage of the 20th day post partum most of the animals showed cyclic activity as it was revealed by the progesterone assays. Therefore an earlier period is likely to be more favourable for inducing cycles in dairy cows to prevent the development of ovarian disorders and improve fertility.

SPECTROFLUORIMETRIC QUALIFICATION OF BOAR SEMEN: A SIMPLE METHOD FOR RAPID ASSESMENT

/T.Takács, J.Matkó, L.Mátyus, S.Papp, J.Szöllősi, I.Resli and S.Damjanovich/
9th International Biophysics Congress, 23-28 Aug. 1987. Jerusalem/

Flow cytometry is a highly effective method for analyzing spermatozoal subpopulations in fluorescently stained semen of different species of domestic animals. The different dual staining methods provide quantitative data on distribution of functional and nonfunctional spermatozoa. The methods offer a possibility of quantitative analysis of spermatozoan viability and potential fertility. The present study aimed at comparison of the viability data /i.e. proportion of intact cells/ obtained by flow cytometry and by steady-state fluorimetry and determination of the suitability of the latter for qualification of fresh and frozen thawed boar semen in field conditions. The spectrofluorimetric examinations were performed in a modified fluorimeter using the propidium iodide-staining method. Simultaneously, aliquots of the semen were also analyzed in a modified Becton-Dickinson FACS III flow cytometer by a dual staining method. Fresh, stored and frozen-thawed samples were examined immediately and after heat-stress. The viability indices determined by the flow cytometric and the steady-state fluorimetric procedures, using different markers, showed a good correlation in all cases. Therefore, the simple fluorimetric method seems useful for rapid assessment of semen quality in any phase of preservation.

It may be a reliably tool for an objective test of the ejaculates and the preservation technique in artificial insemination laboratories.

INDUCED GYNOGENESIS ON EUROPEAN CATFISH /SILURUS GLANIS L./

/Z.L.Krasznai and T.Márián/
Proceedings of the World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture, Bordeaux, 27-30 May, 1986. Vol.II. Berlin, 1987.

Diploid gynogenesis of European catfish was introduced by cold shock. Sperm was inactivated with Co^{60}, 100 Gy /100,000 rad/ of radiation. The duplication of the maternal genome was induced by cold shock at +4°C for 30 minutes. The optimal onset of the cold shock was 5 or 35 to 45 minutes after fertilization.
By using this method, 20 to 30 % viable gynogenetic diploid offspring can be obtained. To detect gynogenesis, serum esterase marker was used.

INTERSPECIFIC HYBRIDIZATION OF WARM WATER FINFISH

/Z.L.Krasznai/
Proceedings of the World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture, Bordeaux, 27-30 May, 1986. Vol.II. Berlin 1987.

In the class of Pisces, in contrast to other vertebrates, the occurrence of viable natural and artificial hybrids is a common phenomenon. Since the turn of this century, hybridization of fish species has increasingly provided new and significant results in both research and farming. Enormous numbers of different species, 20 000 to 25 000 have been included out of the existing 50 000 /DENTON, 1973/.

The spread of the artificial propagation technique has made more crossing and hybridization work possible. No accurate data are available concerning artificial and natural hybrids, but their number can be estimated at several thousand, or about 5000 to 6000 /SCHWARTZ, 1972, 1981; CHEVASSUS, 1979/, and there is an unlimited potential in this field. With slight exaggeration, we can state that it is merely a technical problem to develop a new "product", e.g. hybrid, with genetic manipulation. Close genetic relationship naturally renders the possibility of successful crossing more feasible. Intespecific hybridization can be regarded as crossing between species or "heterospecific insemination" /CHEVASSUS, 1983/ though some scientists call intraspecific hybrids the crosses of wild and domestic strains of species.

Numerous scientific and practical results can be expected from interspecific hybridization. The following are some of the most important aspects:

- Combination of different genetic features
- Development of new properties
- Changing etology
- Developing new feeding habits
- Producing new heterosis hybrids to increase productivity
- Enlarging the structure of the fauna
- Producing infertile stock
- Development of monosex populations
- Inducing polyploidy
- Inducing gynogenesis or androgenesis.

CORRELATION BETWEEN THE pH VALUE OF UTERINAL DISCHARGE IN DONOR COWS AND VIABILITY OF THEIR EMBRYOS

/J.Bencze and J.Mászáros/
Magyar Állatorvosok Lapja, 1987. Vol.42. No.11. p.653-655.

Studies were carried out in 11 cattle stocks in 51 donors. The pH value of PBS, used for flushing, was determined after storage at +4°C for 24 hours. Viable embryos were obtained only from donors when the pH value of their recovered flushing fluid was between 7.16 and 7.48. The pH value of recovered PBS showed a close correlation /r=0.881/ with the percentage of viable embryos.

HUNGARIAN PROGRAMME FOR EUROPEAN CATFISH AND CYPRINDS

/T.Márián/
Proceedings of the World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture, Bordeaux 27-30 May, 1986. Vol.II. Berlin 1987.

Fish culture and farming play an important role in producing a food supply for mankind. In the last twenty years, freshwater fish culture has rapidly developed. Several new methods of production have been introduced, as a result of which fish production of 2 to 5 tons per hectare could be reached. Specialization in fish breeding has started. Modern fish farms have been established for reproduction, fry rearing, and commercial production. Environmental conditions for fish breeding have significantly improved, but at the same time, the cost of production has been increased. Consequently, fish, as one of the basic factors of production, have become a focus of research work. Finding species and varieties to make production more economical through greater genetic productivity is a main topic of research.
The importance of genetic research and selection has increased. Cultivated carp has become a domestic animal with good adaptability to the environment, and it is easy to modify with genetic and selection methods. Among other cultivated species, selection and genetic study of the European catfish, Silurus glains L., have begun in Hungary during the last few years, and selection of Chinese carps is also planned.

CRYOPRESERVATION OF EUROPEAN CATFISH /SILURUS GLANIS L./ SPERM

/T.Márián and Z.L.Krasznai/
Proceedings of the World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture, Bordeaux, 27-30 May, 1986. Vol.II. Berlin 1987.

Cryopreservation of European catfish /Silurus glanis L./ sperm has been employed to ensure a reliable seed material supply. Sperm was acquired from testes removed surgically.

The effects of Alsever /AL/ as a diluent and two cryoprotectants, dimethylsulfoxide /DMSO/ and ethylene glycol, on the viability of the sperm was tested. Best results were obtained with a 15 % cryoprotectant solution in Alsever. The optimal ratio of sperm: dilutant with 15 % cryoprotectant was in the from range of 1:1 to 1:5. Sperm is rather sensitive to rapid cooling. Best results were obtained by gradual cool-

ing 10°C per minute to -80°C and 20°C per minute from -80°C to -150°C. Liquid nitrogen was used for freezing. The optimal ratios of frozen to thawed sperm in the activating solution were 1:10 or 1:20.

Fertilization tests with cryopreserved sperm demonstrated results nearly as good or the same as the controls. Fertility was 60 to 97 %, and hatching, 40 to 95 %. Fresh European catfish sperm can be stored at +4°C for 48 hours without any impairment of its quality.

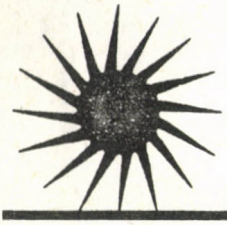
OPPORTUNITIES OF USING BIOTECHNOLOGY IN ANIMAL PRODUCTION

/T.Gere/
Állattenyésztés és Takarmányozás, 1987. Vol.36. No.2. p.115-116.

The author suggests new definition for determination of biotechnology used in the agriculture. Most elaborated method of biotechnology for immediate practical use is the early micromanipulation of embryos of mammals.

Survey is given on opportunities of sex determination of offsprings. Separation of sperms bearing X or Y chromosomes by using monoclonal H-Y antigenes suggest new perspective.
Use of hybridoma produced monoclonal antibodies in animal production is dealt with. Detailed survey is given in respect of perspectives of gene manipulation, first of all efforts directed to change the gene that control growth hormone production.

Strategic concepts of the home biotechnological research and development is detailed in the conclusive part of the paper.



BIOMASS ENERGIES

EXPERIENCES ON THE PRODUCTION AND UTILIZATION OF BIOGAS

/Gy.Bánházi/
Energia és Atomtechnika, 1987. Vol.40. No.2. p. 71-75.

In a survey on the recent international developments, the theoretical research into the kinetics of generation and the effect of the feedstock on the composition of biogas as well as the performance of about 300 production units /mostly in family farms/ in FRG; the practically identical experiences in Italy; the special high-scale plants on left-over food in military barracks in Egypt; the cold procedure used by the Indian and Chinese smallholders; and some data from Rumania and from the GDR are mentioned.
In Hungary, the first large-scale plant /with working volume of 1600 m^3/ is continuously in operation upon the Austrian BIMA system using the 60 m^3 of daily manure yielded at the Co-operative "Rákóczi" at Szécsény. The trial operation is proceeding at the Co-operative "Dózsa" at Dömsöd with the over-all effective volume of 2300 m^3 /divided into 4 units of 575 m^3 each/ on the basis of a dairy-farm holding 1000 cows, processing pig manure and faecal matter besides the stable-litter. The third plant is being builts at the Co-operative "Kőrösmenti" at Szeleste upon poultry-litter with 6 units of 480 m^3 each. The few number of small-sized units are installed at smallholders and dumping grounds.
The future prospects and plants for further developments are also covered.

THE PROSPECTS OF THE UTILIZATION OF BIOMASS

A biomassza hasznositásának távlatai
/Láng I./
Közgazdasági Szemle, Vol.33. no.5. 1986. p.513-520.

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THE POSSIBILITIES OF THE USE OF THE BIOMASS

A biomassza hasznositásának lehetőségei
/Láng I./
Mezőgazdasági Könyvkiadó, 1985. p.249.

The energy crisis broken out at the beginning of the seventies emphasized the utilization of the plant products for genetic purpose. The conscious economy with the renewable power sources has gone to the forefront of the interest. In this topic the utilization of the by-products and waste materials are of significant importance.

SUMMARY OF THE REVIEW ARTICLES FROM BIOTECH-INFO ISSUED IN 1987 and 1988

1987.
No. 1.
Biotech '86. International Conference and Exhibition on Biotechnology, London, 13–15th of May, 1986.
The conference was organized by the Online Int. Ltd. The program and the lectures are reviewed.

No. 2.
The application of anther culture method in crop improvement of Switzerland
The preparation and breeding of nutrient, the assessment of the method, the integration of anther cultures in the practical improvement of corns are presented.

No. 3.
Biotechnology in the pharmaceutical industry. I. The role of biotechnology in human health care
Human medicines and health care products prepared by biotechnology are reviewed. The medical preparations of microbial, aminal origin, gene manipulated compounds, vaccines. The function of chemical synthesis. Transport of medicaments and the biotechnics. rDNA-technique and the economical aspects of the question.

No. 4.
a) **Biotechnology in the pharmaceutical industry. II. Role of biotechnology in the animal health care**
Animal health care products: vaccines for farm and domestic animals, market situation.
b) **Gene transfer in Salmo gairdneri Rich by microinjection into the roe cytoplasma**
A plasmid, containing human growth hormone was injected into the roe cytoplasma. The method and the results are presented.

No. 5.
Bioelectronics. Part II. Biological background of bioelectronics
Molecular engineering: microtechniques and biological models. Biomolecules in electronic devices. Possibility of gene manipulation and protein engineering. Biocircuits. Research institutes dealing with this problem.

No. 6.
a) **IIIrd European Conference on Industrial Microbiology, 23–24, October, 1986. Milano**
Report on the conference proceedings.
b) **Biotechnology and aspects of warfare.**
Biological military materials, weapons, regulation of the biotechnology transfer.

No. 7.
a) **Plant tissue culture in Finland.**
Report about a study-tour in some Finnish research institutes.

b) **Biotechnology program in Poland.**
Coworkers of the Hungarian Academy of Sciences give a review on the situation of the "Central Biotechnological R+ D Program" in Poland.
c) **Potato as a model for plant tissue culture.**
History of the plant tissue culture in general, use of potato as a model.

No. 8.
Exchange of information on biotechnology and genetic engineering.
Bad Ischle, Austria, February 1987.
The main topics were the following: genetic engineering in the basic research, in plant breeding, in the medicine; industrial procpects of the biotechnology.

No. 9.
a) **IV. International Symposium on Feed Physiology.** Czechoslovakia, 22–25. April, 1987.
Review of the subjects referred to.
b) **Techniques for the production of transgenic livestock.**
Material about the 37th Annual Meeting of the European Asscociation for Animal Production, Budapest, Hungary, 1–4. September, 1986.
c) **In vitro fertilization (IVF) of bovine ovule: Effect of condition of medium and donors on the IVF proportion.**
Presentations from the mastercourse organized by the Budapest Veterinary University, 10–11. November, 1986.
d) **Biotechnology in animal product manufacturing.**
Rewiew of five articles in the given field.

No. 10.
a) **Hybride antibiotics – the contribution of the new gene combinations.**
Prospects of genetic engineering in the pharmaceutical industry.
b) **Biodegradable carriers for the sustained release of polypeptides.**
Information about the biodegradable polymers for dosage of pharmaceutical products.
c) **Immunoaffinity chromatography of clinical products.**
Purification of antigenes with a new method – immunoaffinity chromatography.

No. 11.
a) **National policies towards biotechnology.**
Science policy in the USA and the European countries in the field of biotechnology.
b) **New hope of false promise? – Biotechnology and Third World agriculture.**
Review of the International Coalition for Development Action, Publ. 1987. with the remarks of a Hungarian research scientist.

No. 12.
a) **Application of gene recombination technology in plant breeding.**
Preparation of vectors, isolation of genes for plant breeding.
b) **Assessing cell-free translation for protein production.**
Possibilities for application of cell-free systems in the mass production of protein. Advantages and disadvantages.
c) **György. P. Rédei: Genetics**
Review of an excellent Hungarian book on genetics.

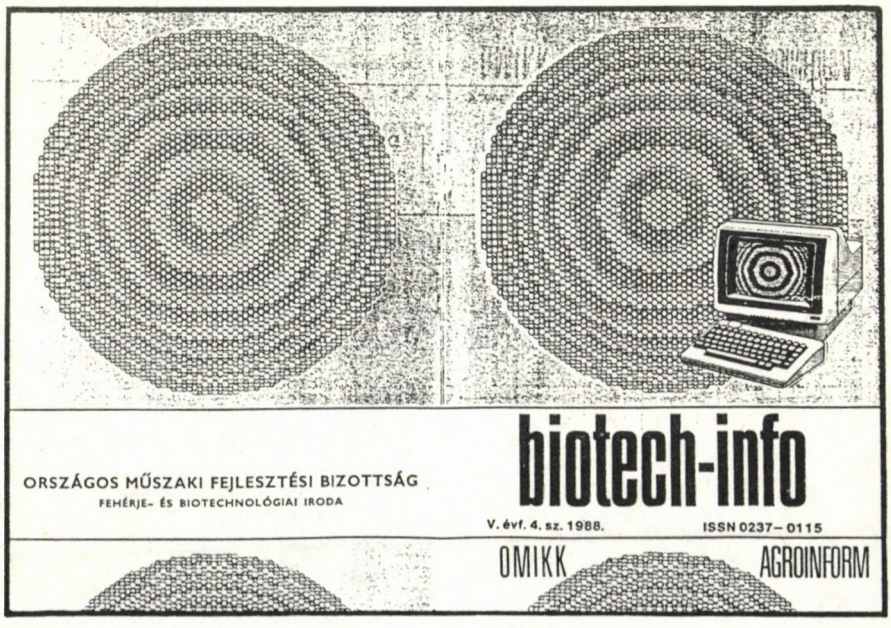
1988.

a) **ICGEB workshop "Biotechnology and industrial commodities".**
Triest, 3– 7 March, 1986.
Abstracts of the lectures of the conference.
b) **4th European Conference on Biotechnology.**
Amsterdam, 14– 19 June, 1987.
The program of the conference is presented.
c) **International Symposium: Biotechnology at the Theshold of the 21th century.** 15– 18 October, 1986. Tokyo, Bioindustry Development Centre
The topics of the symposium are reviewed.
d) **10th Congress of the Hungarian Microbiological Assocciation.** Szeged, 26– 29 August, 1987.
Titles and abstracts from the material of the congress.
e) **Biotechnology Bulletin (in Hungarian: Biotechnológia Füzetek).**
The series titled Biotechnology Bulletin deals with the problems and publications on biotechnology in the South region of the great Hungarian Plain.

No. 2.
a) **3rd International Analytical Symposium of Steroids.** Sopron, 20– 22 October, 1987.
Abstracts of the lectures of the symposium.
b) **Bioreactor Engineering Course.** 4– 11 October, 1987. Ljubjana
The first aim of the course is the formation of young Yugoslav specialists in bioengineering.
c) **Plant biotechnology as a tool for research and production.**
Report on the activity of the Nestlé firm in this field of the biotechnology.

No. 3.
Application of genetic engineering in the plant and animal breeding.
Recent developments in these branches of the economy are presented.

No. 4.
List of periodicals abstracted in Biotech-Info.



Economic background of biotechnology in the capitalist countries. p. 7– 50. in: **ECONOMIC ASPECTS OF BIOTECHNOLOGY.** (TD151), OMIKK, Budapest, 1987. p. 240 (Hungarian language) **STUDY**

On the basis of a two year monitoring of economic topics we compiled a publication titled "Economic aspects of biotechnology". After a rough systematization of the material we have found the following topics to be worth processing:

1. Role of high-tech firms and scientific/industrial parks in biotechnology.
2. University/industry relationship and the role of governmental support in biotechnology.
3. Various models in development of biotechnology. (USA– Japan– Europe)
4. Types of firms in biotechnology.
5. World economic background and the financial environment, economic assesment of processes, role of venture capital and new venture forms.
6. Scale-up and downstream processing in biotechnology.
7. Questions of education, laborforce and the technology transfer in biotechnology.

We dare to say that the greatest part of topics under the title biobusiness contains the economic background of biotechnology, organizational and enterprising criteria, regulations, financial sources and there are only little expensive and hardly accessible information about the output, costs or scale-up data of products or technologies. This is the most jealously guarded information of the biotechnology companies.

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The National Technical Information Centre and Library, OMIKK has developed from a small special library, founded in 1883, to the largest scientific and technical information centre and library in Hungary.

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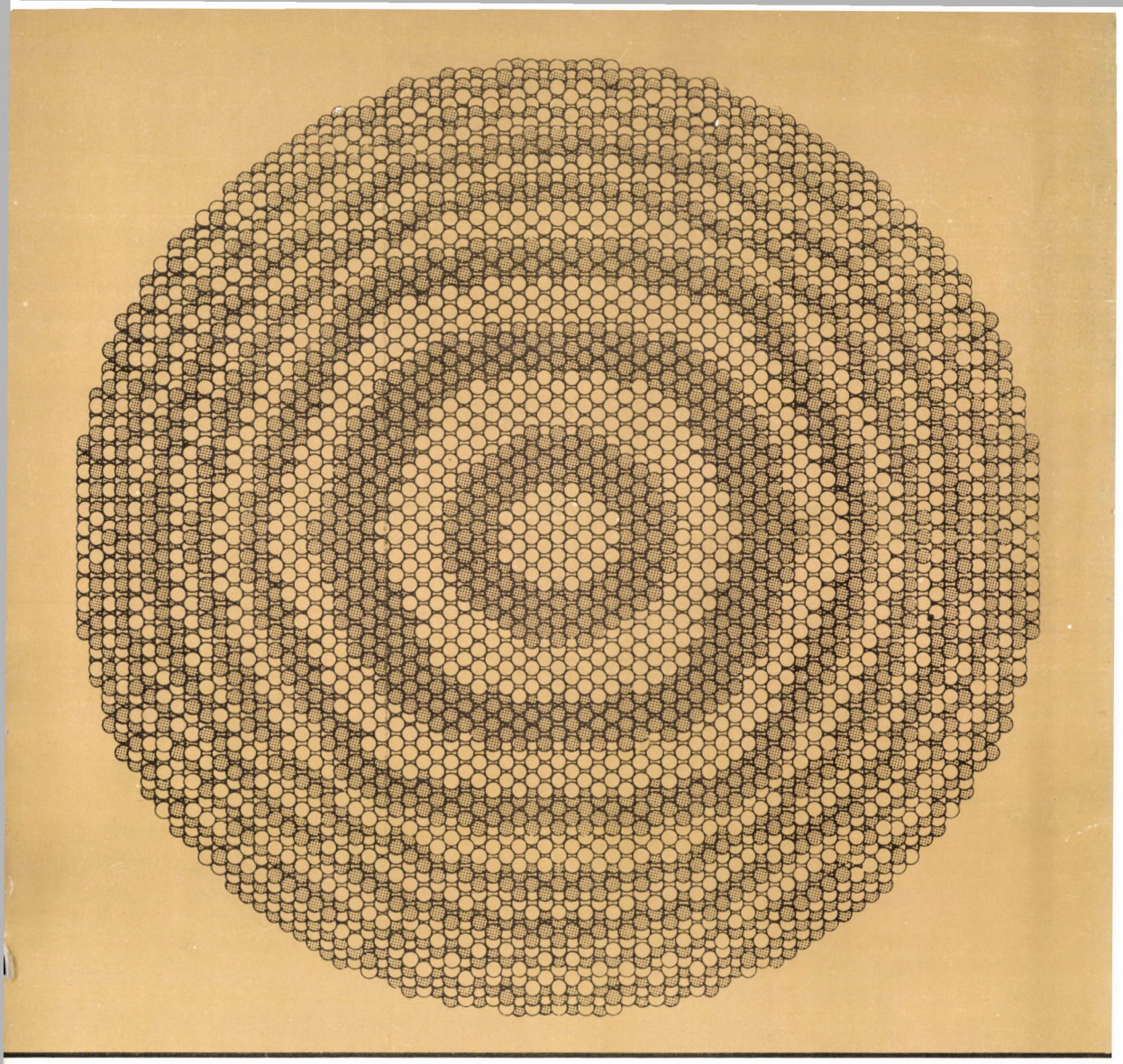
This brochure aims to give a brief but still overall picture of the Institution, its organization and services, thus making you acquainted with Hungary's largest scientific and technical information centre and library — rich in tradition and new achievements.



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