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Biochemistry

IDENTIFICATION OF *DIOSCOREA BATATAS* (DECNE) TUBER PROTEINS

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(Received April 14, 2004)

Abstract

Tuber proteins of *Dioscorea batatas* were purified by hydrophobic chromatography on Phenyl-Toyopearl 650M and anion exchange chromatography on HiTrap Q. Major storage proteins accounted about 70% of total protein content and were identified by SDS PAGE and N-terminal amino acid sequencing. Storage proteins were composed of a single 31 kDa polypeptide and revealed high homology to dioscorins from other yam species. Another isoform accounted *ca* 20% of the total protein content and was heterodimer of 66 kDa and 31 kDa subunits with inter-chain disulfide bonds in the large subunit. N-terminal amino acid sequencing of minor tuber proteins showed homology to *Galanthus nivalis* agglutinin and yam acidic chitinase.

Key words: *Dioscorea batatas*, dioscorin, storage proteins, yam chitinase.

Abbreviations: DB - *Dioscorea batatas*; EDTA - ethylenediamine tetraacetic acid; HPLC - high-performance liquid chromatography; SDS-PAGE - sodium dodecylsulfate-polyacrylamide gel electrophoresis.

Introduction

Yams, *Dioscorea* spp., are one of the oldest carbohydrate food crops and are grown for their tubers. Yam tubers are basically made up of carbohydrates, but also constitute an important source of proteins. Studies on tuber proteins in various yams revealed that they contain *ca* 1-3 % crude protein as eaten and 6-13 % when measured on a dry wt basis [Coursey D., 1967, Muzac-Tucker I., et al., 1993]. The vast majority of tuber proteins account about 85 % of the total protein content and defined as major storage protein dioscorin [Harvey P., Boulter D.,1983]. Dioscorins, are mainly allocated in vacuoles and tend to be associated in high molecular weight polymers depending on milieu conditions [Conlan S., et al., 1998, Harvey P., Boulter D., 1983].

In the present work we identified tuber proteins from the widely distributed yam specimen in northern Japan *Dioscorea batatas* and described properties of major storage proteins in regard to subunit organization and relation to other tuber proteins.

Materials and Methods

Yam tubers were obtained in northern Japan and stored at 4°C until use. Tubers were homogenized in equal volume (v/w) of 0.05 M sodium acetate buffer pH 4.0. The homogenate was centrifuged at 15, 000 ×g for 40 min. The supernatant was adjusted to pH 7.0 with 6 M NaOH and ammonium sulfate was added to give 25 % final saturation. Extract was centrifuged at 15, 000×g for 40 min and supernatant was applied to hydrophobic chromatography on a Phenyl-Toyopearl 650M column (3.5 x 20 cm) (Tosoh, Japan) equilibrated with 0.05 M Tris-HCl buffer (pH 7.0) containing 25 % ammonium sulfate. Elution was performed by gradient decrease of ammonium sulfate concentration from 25 % to 0 %. Then, protein fraction was applied to an anion-exchange chromatography on HiTrap Q column (5 ml) (Amersham Pharmacia Biotech., Sweden) equilibrated with 0.05 M THB (pH 8.0). Elution was performed by gradient increase of NaCl concentration to 0.3 M.

Gel-filtration of the purified proteins was performed on HiLoad 16/60 Superdex 200 prep grade column (1 x 30 cm) (Amersham Pharmacia Biotech.) using 8 M urea in 0.05 M TBS (pH 8.0) as an elution buffer and TSK 3000G SW column (7.5 x 30 cm) (Tosoh, Japan) equilibrated with 0.25 M sodium phosphate buffer (pH 7.0).

For amino acid sequence of NH_2-terminal domains, purified proteins were reduced with 10 mM dithiotreitol in 0.25 M Tris-HCl (pH 8.6) containing 10 mM EDTA and 6 M guanidine hydrochloride and incubated at 37 °C for 2 h. Then, reduced monomers were reacted with 0.02 M iodoacetamide for 20 min at room temperature in the dark. Excess reagent was removed by dialysis against distilled water and lyophilized. Reduced and carboxamidomethylated proteins were directly applied to N-terminal sequencing on a protein sequencer (PPSQ-10 Shimadzu, Japan). In some experiments, proteins were transferred electrophoretically to Trans-Blot PVDF membrane (BIO-RAD) by the method of Laurriere [Lauriere M.,1993]. PVDF membrane was stained with 0.1 % Coommasie Blue R-250. The protein bands were cut out, destined and subjected to N-terminal sequencing. Homologous sequences were searched by FASTA program accessed by Genome Net WWW. SDS-PAGE was performed in 15% (v/w) acrylamide gels as described by Laemmli [Laemmli U.,1976]. Non-denaturing PAGE of native proteins was performed according to the Davis [Davis B.,1964]. Protein concentration was estimated by BCA assay (BIACORE) using bovine serum albumin as a standard.

Results and Discussion

Extraction of tuber proteins with sodium acetate buffer followed by hydrophobic chromatography on Phenyl-Toyopearl 650M allowed to recover *ca* 2 g of total protein per 1 kg of fresh tuber. Anion-exchange chromatography on HiTrap Q HP yielded four distinct peaks designated as DB1, DB2, DB3 and DB4, respectively (Fig. 1). DB2, DB3 and DB4 were retarded proteins and accounted *ca* 50 %, 15-20 % and 8-10 % of total tuber protein content respectively (Table 1). DB1 was flow-through protein fraction and gave two polypeptide bands of 20 kDa and 10 kDa upon SDS gel electrophoresis, but only one band of 10 kDa under reduced conditions (Fig.2, lanes 1, 2). DB2 and DB4 gave single bands of 31 kDa and 28 kDa respectively on SDS-PAGE without 2-mercaptoethanol treatment (Fig. 2, lanes 3, 4) and single bands of 31 kDa and 33 kDa under reduced conditions (Fig. 2, lanes 5, 6). DB3 yielded two polypeptide bands of 66 kDa and 31 kDa and a single band of 31 kDa under reduced conditions (Fig. 2, lanes 7, 8), indicating the presence of inter-chain disulfide connections within 66 kDa polypeptide. Upon gel-filtration chromatography on HiLoad 16/60 Superdex 200 and TSK 3000G SW DB3 complex eluted as a single unit revealing no association-dissociation reaction between subunits depend on protein

concentration, pH value and ionic strength. Complete separation of monomers, nevertheless, was achieved only in the presence of denaturing agents (8M urea) in the elution buffer.

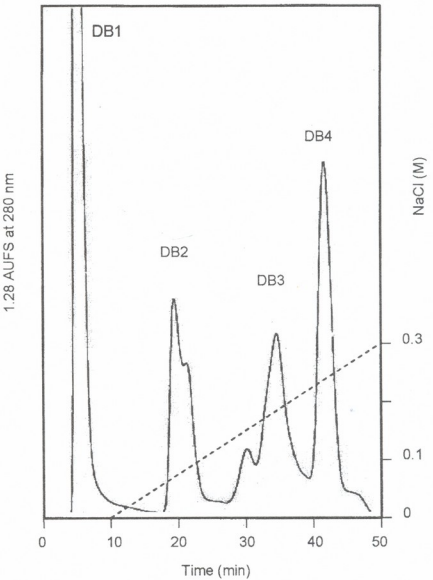

Fig. 1. Purification of yam tuber proteins on HiTrap Q HP column. Elution of proteins was performed by a NaCl gradient from 0 to 0.3 M in 0.05 M Tris-HCl (pH 8.0) at a flow rate of 1 ml/min. Peaks 1~4 were designated as DB1~DB4 respectively. DB1, low molecular weight tuber protein; DB2 and DB3, major storage proteins; DB4, supposed yam acidic chitinase.

Table 1. The yields of *Dioscorea batatas* tuber proteins.

Purification step	Protein (g)[a]	Recovery (%)
Homogenate	2.3	100
Phenyl Toyopearl 650M	2.0	87
HiTrap Q	1.2	53
DB1	0.22	
DB2	0.60	
DB3	0.27	
DB4	0.13	

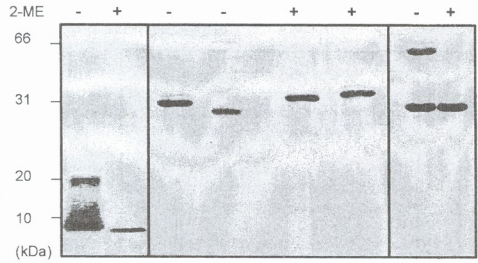

Fig. 2. SDS-PAGE of yam tuber proteins in 15 % acrylamide gels. Lanes 1 and 2 are non-reduced and reduced DB1, respectively. Lanes 3 and 4 are non-reduced and reduced DB2 and DB4, and lanes 5, 6 are reduced DB2 and DB4, respectively. Lanes 7 and 8 are non-reduced and reduced DB3, respectively. Bovine serum albumin(66, 000), ovalbumin(45, 000), carbonic anhydrase (29, 000), soybean trypsin inhibitor (21, 500), cytochrome C (13, 000) were used as standard markers.

NH_2-terminal domains of DBs were determined by applying of carboxamidomethylated proteins to sequential Edman degradation. The first 25 amino acids in N-terminal region of 31 kDa polypeptides of DB2 and DB3 showed the similar sequences: Val-Glu-Asp-Glu-Phe-Ser-Tyr-Ile-Glu-Gly-Asn-Pro-Asn-Gly-Pro-Glu-Asn-Trp-Gly-Asn-Leu-Lys-Pro-Glu.The N-terminal region of 66 kDa polypeptide of DB3 respectively was: Asp-Glu-Asp-Asp-Phe-Ser-Tyr-Ile-Glu-Gly-Ser-Pro-Asn-Gly-Pro-Glu-Asn-Trp-Gly-Asn-Leu-Asn-Pro-Glu-Trp.Homologous sequences searched by FASTA program revealed high homology between NH_2-terminal domains of DB2 and DB3 subunits and deduced amino acid sequences of dioscorin from *D. cayenensis* L. cDNA [Conlan S. et al.,1995] and to the dioscorins A and B obtained from cDNA clones of *Dioscorea alata* L. [Hou W.C. et al., 1999] (Fig. 3).

```
DB1(1-24)                                  DFILYSGEBLRSGQALTHGSYTFI.....
GNA(1-47)     ----MAKASLLILTTIFLGVITPSCLSENILYSGETLPTGSSLTSGSFVFI.....

DB2 (1-24)                                 VEDEFSYIEGNPNGPENWGNLKPE.....
DioA(1-51)    MSSSTLLHLLLLSSLLFSCLPNAKPQQAEDEFSYIEGNPNGPENWGNLKPE.....

DB3L(1-24)                                 D-EDDFSYIEGSPNGPENWGNLNPE.....
DioB(1-50)     MSSSTLFHLFLLSSLLFSCFSNARIDGDDDFSYIEGSPNGPENWGNLRPE.....

DB3S(1-24)                                 VEDEFSYIEGNPNGPENWGNLKPE.....
DioA(1-51)    MSSSTLLHLLLLSSLLFSCLPNAKPQQAEDEFSYIEGSPNGPENWGNLKPE.....

DB4(1-24)                                  QNCQCDTTITCCSQHGYCGNSYDY.....
Cht(1-24)                                  QNCQCDTTIYCCSQHGYCGNSYDY.....
```

Fig. 3. Comparison of N-terminal amino acid sequences of *D. batatas* tuber proteins and homologous proteins from other plants. Amino acid sequences are aligned to maximize similarity. The

identical amino acids are boxed. DBs: *D. batatas* tuber proteins, DB3L: 66 kDa subunit of DB3, DB3S: 31 kDa subunit of DB3. GNA: *Galanthus nivalis* agglutinin. DioA: dioscorin A from *D. cayenensis*. DioB, dioscorin B from *D. alata*. Cht, acidic chitinase from *D. japonica*.

Thus, storage proteins from *D. batatas* revealed high homology to those of dioscorins from different yam species. In *D. batatas* storage proteins were obviously presented in two isoforms: free form (DB2) was composed of a single 31 kDa polypeptide as indicated by SDS-gel electrophoresis and accounted *ca* 50 % of the total protein content. Another form of storage proteins (DB3) accounted *ca* 15-20 % of the total protein content and was a complex of two subunits of 66 kDa and 31 kDa with inter-chain disulfide bonds in the large subunit. N-terminal sequence analysis showed significant differences between 66 kDa and 31 kDa polypeptides. Particularly, Val, Glu, Asn and Lys residues in the positions 1, 4, 11 and 22 in the 31 kDa subunit were respectively substituted by Asp, Asp, Ser and Asn in 66 kDa subunit, indicating that protein complex is composed of different polypeptides and is not a result of non-covalent interactions between 31 kDa subunits [Conlan S., et al., 1998, Harvey P., Boulter D.,1983, Hou W.C., et al.,2000]. In addition, both subunits were constantly presented in the tuber preparations showing significant stability upon the variations of milieu.

The small polypeptides (10 and 20 kDa) of DB1 were electroblotted onto a PVDF membrane and subjected to the protein sequencer. The first 24 amino acids in the NH_2-terminal domains of both polypeptides gave similar sequences: Asp-Phe-Ile-Leu-Tyr-Ser-Gly-Glu-Ser-Leu-Arg-Ser-Gly-Gln-Ala-Leu-Thr-Arg-Gly-Ser-Tyr-Thr-Phe-Ile. SDS PAGE and N-terminal sequence analysis indicated that, newly detected protein obviously does not belong to dioscorin family, however, shows homology to deduced amino acid sequence of mannose-specific agglutinin from *Galanthus nivalis* [Van Damme E., et al.,1991]. Both polypeptides of DB1 yielded the same N-terminal amino acid sequence. Supposedly, the protein is a mixture of two isoforms with molecular masses of about 10 kDa capable to form dimmers due to the presence of extra cysteine residue in one polypeptide.

The N-terminal sequence analysis of DB4 yielded following sequence: Gln-Asn-Cys-Gln-Cys-Asp-Thr-Thr-Ile-Thr-Cys-Cys-Ser-Gln-His-Gly-Tyr-Cys-Gly-Asn-Ser-Tyr-Asp-Tyr.
Homologous sequences searched by FASTA program revealed high homology to acidic endochitinase (EC 3.2.1.14) from *Dioscorea japonica* with calculated molecular mass of 27, 890 Da [Araki T., et al., 1992]. Yam acidic class I chitinase from *D. japonica* was shown constituently presented in yam tubers and was not induced by an elicitor or pathogens [Araki T., et al., 1992, 1992, 1995].

Thus, yam tubers of *Dioscorea batatas* contain variety of proteins with the vast majority of well-defined major storage proteins, which accounted *ca* 70-75% of total protein content and reveal homology to dioscorins from other yam species. Interestingly, all tuber proteins share the similar features in terms of forming higher molecular weight polymers via disulfide bridges or non-covalent aggregation. In this respect, tuber proteins of *Dioscorea batatas* resemble the storage proteins from other yam species.

Along with the major storage proteins, we detected the presence of new minor components in tuber preparation including newly detected low molecular weight protein DB1 and supposed acidic chitinase. Interestingly, both proteins belong to the defense proteins. Apparently, the tuber proteins of *Dioscorea batatas* are encoded by multiple gene family and the properties of those are not confined only in the passive storage functions.

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DIOSCOREA BATATAS (DECNE) ტუბერის ცილების დახასიათება

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(მიღებულია 14.04.2004)

რეზიუმე

შესწავლილია იაპონიაში ფართოდ გავრცელებული საკვები კულტურის *Dioscorea batatas* (Decne) ტუბერის ცილები. ცილებს ვასუფთავებდით ჰიდროფობული და ანიონ-ცვლადი ქრომატოგრაფიით და ვახასიათებდით გელ-ელექტროფორეზით და N-ტერმინალური ამინომჟავური სექვენირებით. ტუბერის სამარაგო ცილები შეადგენდა ჯამური ცილების 70%-ს და ავლენდა ჰომოლოგიას *Dioscoreaceae* ოჯახის ძირითად სამარაგო ცილა დიოსკორინთან. სამარაგო ცილების მეორე ფორმა შეადგენდა ცილის საერთო რაოდენობის 20%-ს და წარმოადგენდა 66 kDa და 31 kDa სუბერთეულებისაგან შემდგარ ჰეტეროდიმერს შიდაჯაჭვური დისულფიდური ბმებით. ტუბერის ახლადგამოვლენილი მინორული ცილების N-ტერმინალური სექვენირებით გამოვლინდა ჰომოლოგია *Galanthus nivalis* აგლუტინინთან და *Dioscorea japonica*-ს მჟავურ ჭიტინაზასთან.

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Biochemistry

LOCALIZATION AND MECHANISM OF ACTION OF THE COLA PLASMID DNA REGION DETERMINING ITS STABLE INHERITANCE

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(Received April 14, 2004)

Abstract

Two par-regions with sizes 700 and 1100 bp, which ensure plasmid stable inheritance in strain *E.coli C600* are located in a structure of a small colicinogenic plasmid ColA. In strain *E.coli IC 8679* where multimerization is the highest, only one of the fragments ensures the stabilizing effect with multimer resolution. By Southern hybridization in colinicogenic plasmid ColN the par-site homologous with ColE1 was revealed. It is supposed that colicinogenic plasmids stabilization is also determined by functioning of genes, connected with colicine synthesis and its action.

Key words: ColA, plasmid stability, homology, par-site.

Introduction

It is well-known that in natural bacterial plasmids there are DNA regions, determining plasmid distribution into daughter cells at cell division (par-sites) and thus, their stable inheritance in populations [Mann, 1985]. The mechanisms of plasmids segregative stability having both fundamental and applied significance have been intensively studied [Gottfried et al., 2003, Kolot et al., 2003].

One of the most preferable objects of the study of the given problem are small multicopy colicinogenic plasmids [Rekesh et al., 1987]. The plasmids of this group serve as a good model for the study of replication, recombination, transcription and translation mechanisms for many years; their derivatives are used for the construction of various vector plasmids which, as a rule, are instable in nonselective conditions.

Thus, the study of functional organization of small colicinogenic plasmids par-sites allows to elucidate the mechanism of their stabilizing action as well as to use them for recombinant *Escherichia coli* plasmids stabilization.

Present work is devoted to the study of structural-functional organization of DNA regions of a small colicinogenic ColA plasmid, determining its stable inheritance and distribution of ColE1-type par-sites among various colicinogenic plasmids.

Materials and methods

Strains *E. coli* and plasmids, used in the experiments are presented in Tables 1 and 2.

Media, used in the study and methods of transformation, isolation, cutting, with restrictases and ligation of plasmid DNA, electrophoresis of DNA fragments in agarose gel and hybridization according to Southern were carried out as described in manual [Maniatis, et al., 1982]. Determination of the plasmid stability was carried out as described earlier [Meacock and Cohen, 1980].

Table 1. Strains of *E.coli* used in the experiments

Strain	Genotype	Source
C600	$rk^{\cdot}$, $mk^{\cdot}$, leu, thr, thi, str^r	Museum of the Institute of Molecular Genetics, Academy of Sciences of Russian Federation.
JC8679	thr, leu, pro, his, arg, thi, str^r, recB, recC, sbcA	Chermin L.S. Institute of Chemical Physics, Academy of Sciences of Russian Federation.

Table 2. Plasmids, used in the experiments

Plasmid	Markers	Source
pUC19	Amp^r	Museum of the Institute of Molecular Genetics, Academy of Sciences of Russian Federation.
ColA	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColE2	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColE3	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColE4	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColE5	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColE7	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColE8	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColN	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.
ColD	$Col^+ Imm^+$	Pagsley A., Institute of Pasteur, France.

Results and Discussion

Localization of Col A plasmid DNA regions responsible for its stable inheritance.

One of the most significant determinants of stable inheritance of small multicopy colicinogenic plasmids of ColE1 - type, is the DNA fragment, called par-site. Such sites are localized in ColE1 [Summers and Sherratt, 1984], ColK [Summers et al., 1985], CloDF13 [Van Den Elzen et al., 1983] plasmids.

Stabilization of plasmids by means of par-sites of ColE1 type is described by a model, assuming that at cell division a random distribution of multicopy plasmids into daughter cells takes

place. The main reason of these plasmids instability is multimerization, as plasmid multimers exist in cells in less number of copies than monomers.

Par-sites of ColE1-type contain the site which is recognized by a cell enzyme - recombinase, which resolves multimer forms of plasmid DNA into monomers and leads to stabilization of their containing plasmids.

The objective of our study was a small multicopy colicinogenic plasmid ColA. This plasmid was rather well studied: length of ColA – 7000bp, its restriction map is known in details, nucleotide sequence of replication initiation region has been determined, as well as of genes, determining synthesis and action of colicine A.

ColA DNA sites, determining their stable inheritance - par-sites were revealed according to their ability to stabilize the pUC19 vector plasmid, which is very unstable in nonselective conditions of growth of cells containing plasmid. In order to identify par-sites of ColA fragments, this plasmid DNA, received after treatment with restrictase Pst I (Fig. 1) was cloned into plasmid pUC19.

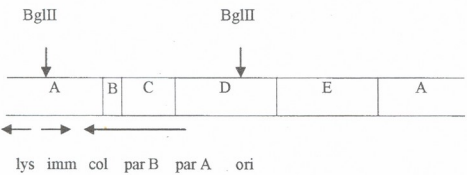


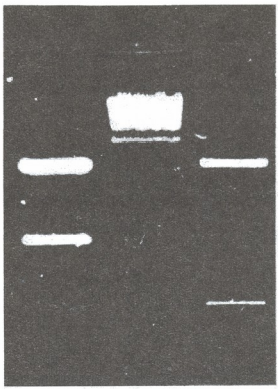
Fig. 1. Genetic map of ColA plasmid: ori – the region of replication initiation; col – colicine A genus; imm – immunity genus to colicine A; lys – lysisis genus; par A and par B – fragments determining ColA stability.

Hybrid plasmids were analyzed on capability to be inherited stable in cells of *E. coli C 600*. The analysis of stability of these plasmids showed that only one plasmid, containing D fragment of ColA (1800bp) is stable in *E. coli* C 600 cells. Issuing from restrictase maps of ColA it was known, that inside of D fragment there is a site of restrictase Bgl II recognising. For a more precise localization of par-site, sub-cloning of separate parts of ColA D-fragment obtained after its treatment with restrictase Bgl II in the pUC19 vector plasmid was carried out.

Recombinant plasmids contained Pst I – Bgl II – fragments of ColA with length 700 and 1100 bp, respectively (Fig. 2). Analyses of these plasmids stability showed, that both of them are stable in *E.coli* C 600. According to Southern hybridization it was demonstrated that par-sites of ColA 700 and 1100 bp were not homologous.

On the basis of obtained data it can be supposed that plasmid ColA has two DNA regions, providing its stability. Preliminary results obtained while the study of two other colicinogenic ColD and ColK plasmids stability indicated on the presence of at least two par-regions in these plasmids, one of which functions according to the mechanism of multimer resolution and another one, probably, stabilizes the plasmid by different mechanism.

Probably there is one more mechanism of stabilization in colicinogenic plasmids, owing to genes presence determining synthesis and action of colicine. Indeed, if at cell division one of the daughter cells appears to be without a plasmid, it would be killed by colicine of another cell containing a plasmid, as it would lose immunity to colicine which is also determined by a plasmid.



1 2 3

Fig. 2. Agarose gel electrophoresis of DNA plasmid containing PstI – BglII – fragment of ColA, 1100 bp (1), PstI – BglII fragment of ColA, 700 bp (3) in the pUC19, treated with restrictases EcoRI and PstI; DNA of phage T DNA, treated with restrictase HindIII (2).

Elucidation of the nature of stabilizing action of plasmid ColA parA and parB fragments.

In order to elucidate the mechanism by means of which ColA par fragments determine their stable inheritance, the hybridous plasmids, containing these fragments were transferred by transformation into strain *E.coli* JC8679. The highest level of multimerization of plasmid DNA was observed in this strain that leads to a sharp decrease of its stability. However, if there is a par-site of ColE1 type in a plasmid, even in case of increased multimerization, it provides an effective resolution of multimers and stability of plasmids [Summers and Sherratt, 1984].

Analysis of stable inheritance of recombinant plasmids containing parA and parB sites in strain JC8679, has revealed definite functional differences between them. It appeared that only parA region provides an effective resolution of multimerous forms of plasmid DNA and stabilization of recombinant plasmid, while parB fragments function less effectively.

The data on functional differences of par-sites of ColA plasmid can be explained in two ways:

1. The stabilizing effect of the both fragments is connected with resolution of multimerous forms of plasmid DNA. However, par A fragment functions more effectively. Probably, it is connected with the fact, that the given region of DNA is more optimum substrate for the enzyme recombinase, which participate in multimer resolution. This difference between parA and parB fragments is observed in strain *E. coli JC8679*, where the degree of multimerization is the highest.

2. It is possible that the stabilizing effect of par B-site is not connected with plasmid multimers resolution and is determined by another mechanism. It can explain a normal functioning of this par-site in *E.coli C600* cells and insufficient effective work in strain *JC8679*.

Distribution of ColEl-type par-sites among various colicinogenic plasmids.

The distribution of par-sites, homogenous to ColE1 par-locus, among various small multicopy colicinogenic plasmids was studied. With this aim hybridization of HpaII-TagI – ColE1 DNA fragment containing par-site with DNA of ColE2, ColE3, ColE4, ColE5, ColE7, ColE8, ColN, ColD plasmids according to Southern was carried out. The results of hybridization conducted in rigid conditions (65^{0}C) are presented in Fig. 3. As it can be seen from the Fig.3a, among all analyzed plasmids, substantial homology with ColE1 par-region is manifested only by plasmid ColN. For a more precise localization of homology region the hybridization of ColE1 fragment containing par-region with DNA of ColN plasmid, treated with different restrictases, on the base of this plasmid restriction map was carried out [Pugsley, 1984]. It was stated that ColN plasmid Kpn I – Pvu II fragment at length 800 bp, develops significant homology with ColE1 par-region (Fig. 3b).

Thus a par-site, manifesting homology with ColA par region is located in ColN plasmid.

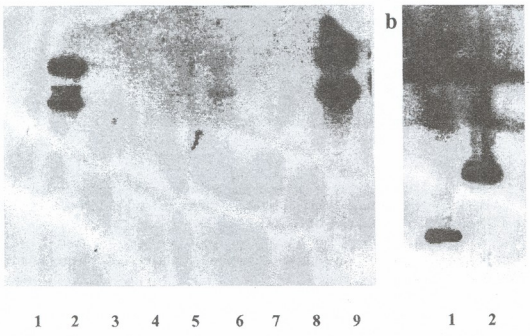


Fig. 3. Hybridization (according to Southern) at 65^{0}C of plasmid ColE1 DNA fragment, carrying par-region: a - with colicinogenic plasmid DNA. 1 – ColE8; 2 – ColN2; 3 – ColE7; 4 – ColE5; 5 – ColE4; ColE3; 7 – ColE2; 8 – ColD; 9 – ColE1. b – with ColN plasmid DNA, treated with restrictases, 1-Kpn I+PvuII; 2 – KpnI.

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ColA პლაზმიდის დნმ-ის სტაბილური მემკვიდრეობის განმსაზღვრელი უბნების ლოკალიზაცია და მოქმედების მექანიზმი

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(მიღებულია 14.04.2004)

რეზიუმე

მცირე კოლიცინოგენური ColA პლაზმიდის სტრუქტურაში ლოკალიზებულია ორი par ფრაგმენტი ზომით 700 და 1100 წყვილი ნუკლეოტიდი, რომლებიც განსაზღვრავენ პლაზმიდის სტაბილურ მემკვიდრეობას *E.coli* C600 შტამში. *E.coli* JC8679 შტამში, სადაც პლაზმიდის მულტიმერიზაციის ხარისხი მაქსიმალურია, სტაბილურობის ეფექტს მულტიმერების დაშლის საშუალებით განსაზღვრავს მხოლოდ ერთი par ფრაგმენტი. ჰიბრიდიზაციის საშუალებით ColN კოლიცინოგენური პლაზმიდის სტრუქტურაში ლოკალიზებულია par ფრაგმენტი, რომელიც ჰომოლოგიურია ColE1 პლაზმიდის par ლოკუსის. კოლიცინოგენური პლაზმიდის სტაბილიზაცია ასევე განისაზღვრება იმ გენების ფუნქციონირებით, რომლებიც განსაზღვრავენ კოლიცინის სინთეზსა და მის მოქმედებას.

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SELECTING THE OPTIMAL CONDITIONS FOR COMBINED SUBMERGED CULTIVATION *OF A. ORYZAE 3–9–15* AND *A. TERREUS AT – 490*

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Abstract

With the purpose of protein – rich biomass gaining, the conditions for combined submerged cultivation of two microscopic fungi – *Aspergilllus oryzae 3–9–15* and *Aspergillus terreus AT - 490* have been established. The nutrient medium with optimal composition and pH, responsible for the maximum output (3g per 100ml nutrient medium) of biomass and high content of protein (45%) was selected. It was determined that the additional sources of nitrogen and phosphorus must be added into the nutrient medium in two stages, so that the final concentrations in the medium were: of $NaNO_3$ 2.2g/l and of KH_2PO_4 – 0.8g/l.

Key words: combined cultivation, biomass, protein, *Aspergilllus oryzae* 3–9–15, *Aspergillus terreus* AT - 490

Introduction

The bioconversion of lignocellulosic substrates into protein – rich biomass is in the center of investigators' attention during last period [Perez et al., 2002, Zervakis et al., 2001, Biljana et al., 2001]. Nowadays wood is considered as a universal lignocellulosic material. Though there also exist other plant substrates which may be converted to improve the deficiency of food stuff protein in stock . Vine cuttings, maize straw, residuals of tea and citrus industry belong to such kind of remains in Georgia.

The main problem is to balance the content of proteins and their elemental amino acids in the fodder. The most popular method of balancing is enriching the fodder with soybean flour. But another way of balancing may be adding of microbial biomass. The remains of food industry, wood making and agriculture may be converted into the food preparations rich of proteins, fats and vitamins [Schulf et al., 1986, Zeltin, 1970].

Materials and methods

The producers of cellulase, thermophilic mutant strains of *Aspergillus terreus AT – 490* and the producer of amylase, mesophilic mutant of *A.oryzae 3 – 9 – 15* were taken, with the purpose of obtaining the protein – rich biomass. These mutant strains were received from the collection of microorganisms of the laboratory of biotechnology of the Institute of Biochemistry and Biotechnology, Georgian Academy of Sciences.

Combined cultivation of micromycetes was performed in 750ml volume cone flasks on the shaker with 180rot/min. At the beginning micromycetes were cultivated on the following medium: (g/l) $NaNO_3$ – 1.9–3, KH_2PO_4 – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.5, KCl - 0.5, $FeSO_4$ - 0.02; pH of the medium was 5.5. Straw of maize or citrus flour served as a source of carbon in the experiments. These plant substrates were previously dried at 55°C, ground and sieved in 1mm diameter sieve. 30g/l of maize straw and 40g/l of citrus flour were added to 100ml of above mentioned cultivating medium and sterilized at 0.7 atmospheres for 45min.

The combined cultivation of fungi was performed following the early elaborated scheme: on the first stage of cultivation the mesophilic mutant strain *A. oryzae 3 – 9 – 15* was grown at 30°C during 48h. On the second stage – another mutant *A. terreus AT - 490* was added into the same flask and the combined cultivation of both micromycetes was done at 40°C for 48h (total fermentation time was 96h).

Results and discussion

As it is known, the growth of microorganisms and protein accumulation in biomass is greatly influenced by the composition and pH of the nutrient medium [Stewart and Parry, 1981]. Our main goal was to select the conditions for the combined cultivation of two mutant strains – *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490*, acting of different polymeric substates and by this way enhance the protein content in biomass. At the beginning of the work we aimed to arrange the nutrient medium for optimal growing of both microorganisms.

The optimization of the nutrient medium was begun by determining the concentration of nitrogen source. Earlier we have concluded that $NaNO_3$ was the best source of nitrogen for submerged cultivation of *A. terreus AT – 490* on maize straw or citrus flour, in concentration not more than 0.5g/l (by nitrogen). $NaNO_3$ was also optimal for *A. oryzae 3 – 9 – 15* in concentration not more than 1.9g/l (by nitrogen). As one and the same source of nitrogen turned to be favorable for both micromycetes, our task was to select the concentration of $NaNO_3$ in the nutrient medium.

In these series of experiment $NaNO_3$ was added (1.3 – 3g/l) to the cultivating medium on the first stage of cultivating scheme, which we've elaborated earlier. We hoped to obtain the high content of protein in the nutrient medium with the concentration $NaNO_3$ 24g/l, as it was the amount of nitrogen source, necessary for the normal developing of both microorganisms (1.9g/l for *A. oryzae 3 – 9 – 15* and 0.5g/l for *A. terreus AT – 490*).

As it is clear from the data (Table 1), none of the used concentrations of $NaNO_3$ appeared to be optimal for increasing the content of protein in biomass.

The combined cultivation of the given fungi on the maize straw or citrus flour resulted in not more than 24% protein producing. It became clear that application of the whole amount of nitrogen source on the first stage of cultivation inhibited the growth not only of *A. oryzae 3 – 9 – 15*, but of *A. terreus AT – 490* too, which was brought in on the second step of cultivation. We changed the scheme of supplying the nutrient medium with nitrogen source and decided to add $NaNO_3$ in two stages. On the first stage of supplying $NaNO_3$ was added to the medium in the amount 1.9g/l, which was the optimal for *A. oryzae 3 – 9 – 15*. On the second stage the source of

nitrogen was additionally carried in the amount 0.3 – 1.1g/l. The results of these experiments are given in the Table 2.

Table 1. Influence of different concentrations of nitrogen source, added at the first stage of cultivation, at mutant strains growth and accumulation of protein in biomass

Substrate	$NaNO_3$ (g/l)	Crude protein (%)	Biomass (g per 100ml of cultivating medium)
Citrus flour	1.9	20	2.0
	2.2	24	2.2
	2.4	20	2.0
	3.0	18	1.9
Maize straw	1.9	18	1.9
	2.2	22	2.0
	2.4	20	2.0
	3.0	18	1.9

From the table it is evident that supplying with nitrogen in two stages stimulated the accumulation of the biomass and enhanced the amount of protein. Mostly interesting seemed the nutrient medium, with the 40 – 42% of total fresh protein in the biomass, diversely from the variant with one – fold adding of the same amount of nitrogen in the cultivating medium, were the amount of protein reached only 22 -24% (Table1)

In early experiments we've determined the optimal concentrations of KH_2PO_4 as the favorable phosphorus source for cultivating both, *A. terreus AT – 490* and *A. oryzae 3 – 9 – 15*. For the first mutant it made 0.5g/l and for the second one – 0.3g/l (by phosphorus).

Table 2. Influence of different concentrations of nitrogen source, added in two stages during the cultivation, at *A. oryzae* 3 – 9 – 15 and *A. terreus* AT – 490 growth and accumulation of protein in biomass

Substrate	$NaNO_3$ (g/l)		Crude protein (%)	Biomass (g per 100ml of cultivating medium)
	I stage	II stage		
Citrus flour	1.9	0.	20	2.0
	1.9	0.3	40	3.0
	1.9	0.5	32	2.4
	1.9	1.1	28	2.2
Maize straw	1.9	0.	22	2.2
	1.9	0.3	42	3.1
	1.9	0.5	34	2.8
	1.9	1.1	25	2.4

In these series of experiments the source of phosphorus was added only once, at the first stage of cultivation scheme, in quantity 0.3 – 1g/l. From the Table 3 it is clear that only one – fold supplying of cultivating medium with KH_2PO_4 didn't give any positive results toward the increasing of protein content in biomass.

Table 3. Influence of the different concentrations of phosphorus source on *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490* growth and accumulation of protein in biomass

Substrate	KH_2PO_4 (g/l)	Crude protein (%)	Biomass (g per 100ml of cultivating medium)
Citrus flour	0.3	18	1.9
	0.5	22	2.1
	0.8	27	2.2
	1.0	25	2.1
Maize straw	0.3	18	1.8
	0.5	20	2.1
	0.8	25	2.2
	1.0	22	2.1

So it was decided to add KH_2PO_4 in two stages. At the first stage of cultivation the substance was added in amount 0.3g/l, which was necessary for the normal developing of *A. oryzae 3 – 9 – 15.* At the second stage of cultivation KH_2PO_4 was pulsed in quantity 0.2 – 0.7g/l. The results of the experiment are given in the Table 4.

Table 4. Influence of the different concentrations of phosphorus source, added in two stages, at *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490* growth and accumulation of protein in biomass

Substrate	KH_2PO_4 (g/l)		Crude protein (%)	biomass (g per 100ml of cultivating medium)
	I stage	II stage		
Citrus flour	0.3	0.	18	1.9
	0.3	0.2	28	2.2
	0.3	0.5	43	3.2
	0.3	0.7	28	2.2
Maize straw	0.3	0.	18	1.8
	0.3	0.2	26	2.2
	0.3	0.5	45	3.2
	0.3	0.7	26	2.2

It is evident that the mutant strains accumulated the maximal amount of biomass in the nutrient medium with total phosphorus content in amount 0.8g/l. The biomass developed in this medium contained 43 – 45% protein.

To arrange the optimal pH of the nutrient medium at the first stage of cultivation, *A. oryzae 3 – 9 – 15* was grown at pH 2.5 – 6.5. On the second stage of fermentation pH of the medium wasn't changed, to avoid the pollution of the cultivating medium. Table 5 demonstrates that mutant strains formed protein – rich biomass on the medium with pH 4.5.

Table 5. Influence of pH of the nutrient medium at *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490* growth and accumulation of protein in biomass

pH of the medium	biomass (g per 100ml of cultivating medium)		Crude protein (%)	
	Citrus flour	Maize straw	Citrus flour	Maize straw
2.5	1.5	1.5	12	15
3.0	1.5	1.5	13	15
4.0	1.9	1.9	25	26
4.5	3.2	3.2	43	45
5.0	2.1	2.1	31	33
6.0	1.9	1.8	24	20
6.5	1.5	1.4	13	14

On the base of above mentioned experiments the optimal composition and pH of nutrient medium was selected for the combined cultivation of *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490.* For the maximal outcome of biomass with high content of protein (43 – 45%), the necessity of two – stage supplying of the nutrient medium with nitrogen and phosphorus sources has been established.

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A. ORYZAE 3 – 9 – 15 – ისა და *A. TERREUS AT – 490* – ის ერთობლივი სიღრმული კულტივირების პირობების შერჩევა

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საქ. მეცნიერებათა აკადემიის ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 22.03.2004)

რეზიუმე

ცილით მდიდარი ბიომასის მიღების მიზნით დადგენილია ორი მიკროსკოპული სოკოს – *A. oryzae 3 – 9 – 15* – ისა და *A. terreus AT – 490* – ის ერთობლივი სიღრმული კულტივირების პირობები. შერჩეულია ოპტიმალური შედგენილობისა და pH – ის საკვები არე, რომელიც უზრუნველყოფს ბიომასის მაქსიმალურ გამოსავალს (3გ/100მლ საკვებ არეზე) და ცილის მაღალ (45%) შემცველობას. დადგენილია, რომ აზოტისა და ფოსფორის წყაროები საკვებ არეში შეტანილ უნდა იქნეს ორ ეტაპად, ისე რომ $NaNO_3$-ის საბოლოო კონცენტრაცია საკვებ არეში შეადგენდეს 2.2 გ/ლ, ხოლო KH_2PO_4-ის – 0.8გ/ლ.

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THREADING POTENTIAL BASED ON THE ORIENTATION OF SIDE CHAINS IN RELATION TO HYDROPHOBIC CORES CENTERS AND ON THE DISTANCE OF RESIDUES FROM GEOMETRICAL CENTER OF PROTEIN GLOBULE

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Abstract

Threading procedures cover a variety of techniques that try to determine whether the sequence of a protein with an unknown structure is compatible with that of a known structure. The orientational angles x^0 of side chains in relation to hydrophobic cores centers and the distance r of residues from the geometrical center of globule are the characteristic of native structure which are used as a basis of threading potentials. Since the correlation coefficient between x^0 and r equal to 0.19 it has been decided that they contain independent information and threading potentials based on both characteristic of native structure will be more effective. This assumption has been examined by the Kruskal-Wallis and threading tests using 73 monomeric protein and 104 multimeric protein subunits. It has been shown that potentials based on combined characteristic highly prevail by efficiency of the ones based only on x^0 orientation.

Keywords: Side chain orientation, distance from geometrical center, statistical analysis, threading potential

Introduction

Many methods have been developed to solve the fold recognition problem. The threading is the most widespread method. The basic idea is to thread the target sequence (sequence of the unknown structure) into the known structure and to evaluate the fitness of sequence for the structure by some kind of environment-based or knowledge-based potentials. On the basis of the statistical analysis of the known protein structures (PDB files), quantitative criteria of protein sequence (1D) - three-dimensional (3D) structure compatibility (3D-1D compatibility) have been

constructed, by which the threading potentials were derived. Using such criteria for each amino acid (or groups of residues), some numerical characteristics describing 3D position or state of the given residue (or groups of the residues) in the native globule can be calculated.

The hydrophobic effect is a dominant force in the process of the stabilization of the native structures of globular proteins. The protein itself is a compactly folded polypeptide chain with a core that is clearly well packed and highly enriched in hydrophobic residues. The protein can be stabilized by more than one group of hydrophobic residues (cores). On the basis of these features of native structure the characters which are connected with the hydrophobic effect and used in fold recognition are derived. For example: solvent accessible surface areas of residues in native fold [Bowie et al., 1991; Kochl and Delarue, 1994], diverse variants of contacts between the residues [Chhajer and Crippen, 2002; Miyazawa and Jernigan, 1996; Samudrala and Levitt, 2002], distance of the residue from the geometrical center of the globule [Sunyaev et al., 1994] etc. are the characters which reflect the affect of the hydrophobic interactions on the native structure of protein.

In the previous work we have shown that the orientation of the C^{α}-C^{β} bond (as the value of an angle x^0 between vectors $C^{\alpha}C^{\beta}$ and $C^{\alpha}T$, where T – is a geometrical center of the core) can be used as a simple criterion (character) for quantitative estimation of 3D-1D compatibility and we have proposed new potential for threading which is based on the side chain orientation [Managadze et al., 2004]. This potential also reflects the influence of hydrophobic interactions.

Our goal is to show whether the combination of the abovementioned characters increase the efficiency of the threading potential.

We think that an effective and simple threading potential will be produced if it is based both on distance and orientation. The relative distance of residue from the geometrical centre of the globule $r_i = R_i / R_{av}$ (Ri as distance of the i-th C^{α} atom from the geometrical centre of globule, R_{av} is average of R_i-s) is the most suitable partner for orientation, since it apparently contains independent information (correlation coefficient between x^0 and r equal 0.19). Using such combined criteria for each amino acid, some numerical characteristics describing 3D position or state of the given residue in the native globule can be calculated. Thus each residue (or type of residues) in the given native structure or in the set of native structures can be characterised by the $F(x^0)$ and F(r) distributions of respectively x^0 and r, roughly speaking independent random variables.

The aim of the paper is to estimate the ability of the combined character to be used as an effective characteristic of the native structure of protein. It means, to show that $F(x^0)$ and F(r) distribution functions, which characterize i-th residue (or group of residues) are independent from the protein architecture and are associated with the general rule of the formation of native structure. Therefore, it is necessary to be convinced, that these functions both for the residues of a certain type and for all residues do not strongly vary from protein to protein, but at the same time, distributions for various types of amino acids differ from each other and from the total distribution for the residues of all types. The reality of the abovementioned events can be estimated by the Kruskal-Wallis (Kendall and Stuart, 1967) and threading tests.

Result and discussion

In Kruskal-Wallis test there have been used 73 (see table 2) monomeric proteins, determined to a resolution higher than 2.0 Å by X-ray analysis, involved in different super family by CATH [Orengo et al., 1997] classification and not being membrane proteins. Proteins, stabilized by a large number of disulfide bonds were not involved in the set of proteins either. In spite of the supposition, that subunit-subunit (domain-domain) interfaces are not the major portion of the subunit (domain) surface, only monomeric, single–domain proteins are considered. Invariability of

$F(x^0)$ was estimated in the previous work [Managadze et al., 2004] and it has been shown that for all residues at α=0.05, distribution functions are invariable. Some groups of residues, among which the invariability of the $F(x^0)$ distributions take place, also are defined.

Results of Kruskal-Wallis test for $F_i(r)$ are shown in table 1. We can see that all residues, excluding Glu, Gly, His, Leu and Ser at α=0.05 show invariability of $F_i(r)$ (i=1,73). At α=0.01, distribution functions for Glu, Gly, His, Leu and Ser are also invariable.

Analyzing data for the pairs of residues, some groups of residues are defined: a) A large group, which comprises six residues - Ile, Leu, Met, Phe, Trp, and Tyr. b) Second group unites together four polar residues - Asp, Gln, Glu and Lys. (These groups correspond to the ones which were defined in the process of the invariability estimation for the $F(x^0)$, Managadze et al., 2004). The rest are not inclined to form any group with some other residues.

The employment of the abovementioned groups increases statistical reliability of the results, which show, that the $F(x^0)$ and F(r) distribution function does not vary from one protein to another for a given group of residues (or particular residues), while one group differs from another in this sense.

For the further test of the validity and usefulness of the proposed characteristics of the native structure, we performed a threading test using 177 proteins (73 monomeric, from the Table 2 and 104 subunit of multimeric proteins from the Table 3. The subunits which stabilize their native structure by disulfide bonds also are a part of the multimeric proteins set). We wanted to answer the question: do the distribution functions corresponding to groups (or particular residues) differ from the distribution function, that characterizes the side chain, independent from the residue type (all residues characteristic).

The test proceed accordingly the method described in [Managadze et al., 2004] and uses scoring function, which is derived from the known structures and has the following form

$$Q = \sum_{m=1}^{n} \left(\ln \frac{p_i^{ax} p_k^{ar}}{q_i q_k} \right)_m \qquad (1)$$

where, $p^{ax}{}_i$ designates the probability (relative frequency) of occurrence of x^0 variable of a-th type side chain in the i-th interval of values and $p^{ar}{}_i$ designates the probability (relative frequency) of occurrence of r variable of a-th type side chain in the i-th interval of values. q_i and q_k are the analogous probabilities (relative frequencies) calculated on the basis of the data of all residues. n is the number of positions (residues) in structure. Suggesting that q_i=0.1 (i=1,10) and q_k=0.1 (k=1,10) and using 73 PDB files (only monomeric proteins), the boundaries of the intervals and $p^{ar}{}_i$ and $p^{ax}{}_k$ probabilities are calculated. The scores are than ranked.

Results of the threading test are given in table 2 for monomeric proteins and in table 3 for multimeric proteins.

As seen from table 2 out of 73 structures, in 72 cases own sequences are ranked first. The own sequence of the 7pti is ranked 2-nd. This protein is small and the deviation of the score from the average of Q in units of s.d. (ΔQ) is less than 4. For considered 73 monomeric proteins the average value of ΔQ equal to 6.6 and prevails analogous value (6.1) which is obtained by the result of threading test for the same set of proteins but with the potentials based only on orientational characteristics of side chains.

Table 3 shows the results of threading test for multimeric protein subunits. Out of 104 structures, in 96 cases own sequences are ranked first. For five protein rank value does not exceed 10 and only for three subunits are more then 10. In the case of these eigth protein subunits, the deviation of the score from the average in units of s.d. (ΔQ) is less than 4. Five subunits in addition to the abovementioned eight, also have ΔQ<4. We can explain this failures: firstly, by the fact that

considering subunits are involved in multimeric complex and therefore have a hydrophobic surface for interaction with the other chain of multimeric complex; secondly, by the fact that some subunits for their stabilization need additional stabilization factors. For example: disulfide bonds as in 1nxb, 1aho, 1mof, 2tgi, 1bx7 and chromophor or metal ions as in 1d2v. Generally, some correlation between domain size and ΔQ is observed.

Thus, threading tests show that potentials based on combined characteristics highly prevail by efficiency of the ones based only on x^0. It is obvious that potentials of combined type are more effective both for monomeric proteins and for multimeric ones and can be boldly used in fold recognition.

Table 1. Kruskal-Wallis statistics H, that examines invariability of distributions of relative distances r_i of residue from geometrical centre of globule[a]

Residue	Statistics H	H_{cr}[b]
ALA	63.685	81.381
ARG	54.057	62.830
ASN	32.166	58.124
ASP	61.732	75.624
CYS	6.603	9.488
GLN	38.032	50.998
GLU	98.048	80.232
GLY	87.978	79.082
HIS	38.214	36.415
ILE	56.611	65.171
LEU	82.988	81.381
LYS	72.583	74.468
MET	18.128	23.685
PHE	43.690	59.304
PRO	58.112	59.304
SER	62.793	61.656
THR	50.214	66.339
TRP	10.849	18.307
TYR	20.279	46.194
VAL	69.496	80.232

[a] $r_i=R_i/R_{av}$ - relative distance, where Ri as distance of the i-th C^{α} atom from the geometrical centre of globule, R_{av} is average of R_i-s.

[b] Critical value of the statistics H_{cr} is given at the significance level $\alpha=0.05$.

Table 2. Positions of native folds in the threading scores' distributions for the set of monomeric proteins

PDB name	Q[a]	ΔQ[b]	Length	Threadings	Rank
1ayx	50.009830	14.105916	492	2091	1
1ald	24.210369	11.069290	363	3972	1
1bhe	29.232201	10.868400	376	3837	1
1cem	39.941929	10.557160	363	3972	1
1a3h	41.389740	9.713023	300	5085	1
2cba	35.483639	9.301049	258	6058	1
5cpa	34.295898	9.180256	307	4966	1
1pud	30.254829	8.567481	372	4244	1
3nul	27.723499	8.553957	127	5285	1
1tml	27.237900	8.515373	286	5393	1
2cpl	28.319210	8.402599	164	3696	1
1knb	30.642469	8.118661	186	3067	1
1cma	23.254070	8.084303	221	2301	1
1tpc	28.452280	7.928415	249	6287	1
3sil	44.287029	7.860104	379	3408	1
1jbc	32.429771	7.838451	237	6630	1
1ako	35.931210	7.825518	268	5794	1
1hfc	23.272341	7.720202	157	4354	1
2plc	32.555222	7.665257	274	5389	1
1azl	15.575830	7.614120	314	4808	1
3pyp	23.669430	7.541011	125	5627	1
1udg	24.496370	7.526201	228	2159	1
1uch	30.392929	7.489062	206	2600	1
1plc	22.811190	7.406793	99	6726	1
1ra9	25.990721	7.354469	159	3998	1
1f3z	23.731140	7.285118	150	5008	1
2cpp	43.730129	7.245027	405	3076	1
1xnb	17.454729	7.140049	185	3275	1
1ifc	19.641350	7.055923	131	5008	1
1bkf	19.955830	6.958701	107	5998	1
5cpv	22.600439	6.843190	108	6057	1
3cla	27.629250	6.767000	213	2453	1
1gox	12.971260	6.733691	350	3818	1
2sns	18.609640	6.684991	141	4732	1
2e2c	23.166920	6.627004	156	4532	1
1a6m	23.591810	6.605162	151	4831	1
1bfg	19.981070	6.579083	126	5466	1
1rgp	22.012341	6.563537	189	2813	1
1pta	12.449130	6.519958	318	4431	1
2a0b	18.532499	6.437787	118	6026	1
1opy	20.096550	6.313606	123	5825	1
1ah7	12.270310	6.293215	245	6405	1
2rn2	19.129000	6.278145	155	4866	1
1158	17.548679	6.210419	164	3696	1
1v39	26.303690	6.198441	291	5274	1
1fus	14.107440	6.100296	106	6161	1
1npk	14.672300	6.040656	150	5008	1
2hts	17.955250	5.976128	88	7239	1
1ris	17.918560	5.969988	97	6924	1
1ctj	12.123960	5.882176	89	7188	1
2cy3	14.181710	5.711725	118	6026	1

1caa	11.847670	5.506997	53	9468	1
2acy	10.815000	5.375738	98	6873	1
1lis	9.641907	5.359005	131	5008	1
1hpi	14.493940	5.356530	71	8137	1
1bgc	8.950378	5.222111	158	4176	1
1ptf	11.551310	5.183239	87	7380	1
1ubq	11.563490	5.060648	76	7818	1
1dhn	9.031409	4.847712	121	6023	1
1orc	10.870920	4.829650	64	8662	1
1shg	11.788450	4.719001	57	9247	1
1ops	12.365830	4.704009	64	8662	1
1rie	9.787424	4.694080	127	5285	1
1dsl	10.928680	4.635701	88	7239	1
1a1x	9.299364	4.588075	106	6161	1
1cei	9.400349	4.576880	85	7580	1
1bd8	-0.749999	4.490280	156	4532	1
1rcd	4.431827	4.448029	171	3302	1
3il8	9.804704	4.367839	68	8406	1
1aba	6.894655	4.365315	87	7380	1
1igd	8.193454	4.354992	61	8989	1
2end	4.213634	4.244435	137	4618	1
7pti	1.610222	3.209259	58	9181	2

[a] Score is calculated by equation (1)

[b] ΔQ is a deviation of the score from the average in units of s.d.

Table 3. Positions of native folds in the threading scores' distributions for the set of multimeric protein subunits

PDB name	Q^a	ΔQ^b	Length	Threadings	Rank
1czf - A	33.217781	12.232490	335	4551	1
1qtv - A	37.171310	10.129800	285	5464	1
1c83 - A	42.285992	9.965796	297	5536	1
1cnz - A	35.635342	9.843911	363	3786	1
1xib	35.848629	9.252295	388	3412	1
1gdo - A	29.821720	9.160390	238	2003	1
1a12 - A	38.236050	8.824779	401	3217	1
1qh5 - A	33.620621	8.809609	260	6048	1
1eon - A	25.487749	8.754903	236	2289	1
1b8o - A	17.955120	8.532699	280	5585	1
2eng	25.667360	8.432061	205	3386	1
1ute - A	31.336439	8.210104	302	4849	1
1qre - A	28.014099	8.045612	210	3076	1
1aun	16.031940	7.935609	208	3110	1
1a28 - A	24.499411	7.919717	251	2078	1
6cel	43.092041	7.918734	433	2786	1
1ql0 - A	23.312950	7.754611	241	6558	1
1bue - A	19.534821	7.689894	265	5679	1
1nbc - A	22.744301	7.475996	155	4954	1
1fkb	19.624969	7.380280	107	7340	1
1kpf	20.961340	7.321549	111	7131	1
4ubp - B	17.496401	7.277131	122	6912	1
3bam - A	23.075020	7.253570	206	3162	1
1kid	18.472839	7.203677	193	3480	1
1dts	20.195770	7.194449	220	2222	1

2arc - A	20.736570	7.148004	161	4144	1
1xgs - A	22.334869	7.106369	295	5862	1
1rey	19.324480	7.101230	130	5888	1
1d4t - A	18.404440	6.949330	104	7710	1
1bsm - A	16.734800	6.895225	201	3470	1
1byq - A	15.308790	6.885496	213	2801	1
1cpo	14.762270	6.881353	298	5225	1
1qb7 - A	19.309441	6.835220	236	2289	1
1a73 - A	12.235840	6.797184	162	3955	1
1qqq - A	15.647910	6.713118	259	6348	1
1lmb - 3	15.593240	6.675294	87	8527	1
1whi	12.988460	6.562656	122	6912	1
1tx4 - A	19.705379	6.551923	196	3591	1
1jpc	16.813311	6.465737	108	7180	1
1mzm	16.835011	6.414408	93	8254	1
1euw - A	14.120120	6.383339	136	4965	1
2rta	14.533470	6.322671	121	7189	1
1ay7 - B	16.565840	6.319283	89	8378	1
1xik - A	17.212351	6.255645	340	4137	1
1qau - A	14.973560	6.127199	112	6942	1
1b8z - A	14.345820	6.038740	67	10031	1
2bop - A	15.271810	6.018830	85	8761	1
9rnt	12.553840	5.854941	104	7710	1
1lit	15.515450	5.802257	131	5328	1
1pcf - A	10.568020	5.733610	66	10189	1
4sbv - A	13.582220	5.709949	199	3516	1
1bkr - A	17.020241	5.659286	108	7180	1
1rzl	12.586490	5.638561	91	8502	1
3lzt	16.038429	5.591167	129	6047	1
1svy	13.805870	5.569508	101	7991	1
1mdc	8.677979	5.524743	131	5328	1
3wrp	15.644160	5.493279	101	7991	1
1dpt - A	11.883310	5.433486	117	7020	1
1kvd - A	5.901904	5.429274	63	10656	1
256b - A	13.434710	5.405723	106	7501	1
2lis - A	11.088710	5.404681	131	5328	1
1ab9 - B	12.446240	5.401132	131	5328	1
1poa	10.208570	5.386965	118	6863	1
1b2p - A	11.432050	5.260031	119	6824	1
1vhh	11.647220	5.213095	157	4580	1
2pii	7.914260	5.147704	112	6942	1
1a70	11.247770	5.110601	97	8441	1
1brf - A	8.839231	5.082081	53	11565	1
1rge - A	12.251260	5.079711	96	8407	1
1vfr - A	9.567015	5.032780	217	2489	1
1puc	12.103400	5.027047	101	7991	1
1bea	9.938192	5.016951	116	7195	1
1kpt - A	4.655808	5.000084	105	7549	1
4ubp - A	10.491840	4.998007	100	8256	1
1bp2	8.973974	4.977411	123	6513	1
1cxy - A	9.946587	4.791917	81	9171	1
1b67 - A	8.058821	4.775978	68	9868	1
3pvi - A	6.527738	4.769099	156	4767	1
1vcc	10.723830	4.679037	77	9495	1

1b3a - A	10.873130	4.677197	67	10031	1
5hpg - A	6.829547	4.631043	84	8929	1
1daz - C	3.601380	4.551624	99	8412	1
1gai	39.988861	4.551612	472	2317	1
1dps - A	6.512045	4.491187	159	4362	1
1dgf - A	15.816750	4.441180	497	2067	1
7rsa	9.410316	4.433251	124	6237	1
1vie	6.120353	4.357434	60	11110	1
1pk4	6.388764	4.137656	79	9331	1
1msi	9.688517	4.127808	66	10189	1
1b0y - A	6.429213	4.114747	85	8761	1
2erl	7.235458	4.095777	40	12699	1
2gn5	4.465357	3.943199	87	8527	1
1nkd	6.615728	3.856247	59	11202	1
1utg	6.603450	3.812173	70	9691	1
1dy5 - A	8.376714	3.811684	123	6513	2
1nxb	5.545377	3.748312	62	10869	2
1bgf	4.072885	3.686354	124	6237	1
1cmb - A	4.658050	3.398656	104	7710	1
1vqb	2.679200	3.313737	86	8686	7
1aho	2.398846	3.053439	64	10500	10
1mof	2.737024	2.900480	53	11565	17
2tgi	1.146287	2.859408	112	6942	9
1bx7	-0.572867	2.461380	51	11715	65
1d2v - A	-5.496953	1.864114	104	7710	250

[a] Score is calculated by equation (1)

[b] ΔQ is a deviation of the score from the average in units of s.d.

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წამოცმის პოტენციალი, ფორმირებული ცილის ნატიურ სტრუქტურაში გვერდით ჯაჭვთა ორიენტაციისა და გლობულის ცენტრის მიმართ დაშორების შესახებ ინფორმაციაზე დაყრდნობით

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(მიღებულია 03.05.2004)

რეზიუმე

წამოცმის პროცედურა გულისხმობს მთელ რიგს მიდგომებს, რომელთა მიზანია შეაფასოს საკვლევი, უცნობი სივრცული სტრუქტურის მქონე ამინომჟავური თანამიმდევრობის ცნობილ სივრცულ სტრუქტურასთან ან დახვევის ტიპთან შესაბამისობა. ცილის ნატიურ სტრუქტურებს ხშირად ახასიათებენ ამინომჟავათა გვერდითი ჯაჭვების ჰიდროფობური ბირთვების გეომეტრიული ცენტრების მიმართ ორიენტაციის x^0 კუთხეთა მნიშვნელობებისა და ცილის გლობულის ცენტრიდან ამინომჟავების დაშორების r მანძილთა მნიშვნელობების განაწილებებით. ნატიური სტრუქტურის ამ მახასიათებლებზე დაყრდნობით ხდება წამოცმის პოტენციალების ფორმირება. რადგანაც x^0-სა და r-ს შორის კორელაციის კოეფიციენტი არის 0.19, ამიტომ ჩავთვალეთ რა რომ ისინი შეიცავენ ერთმანეთისაგან დამოუკიდებელ ინფორმაციას (წარმოადგენენ დამოუკიდებელ სიდიდეებს) ვივარაუდეთ, რომ ერთდროულად ორივე ზემოთმოთხსენებულ მახასიათებელზე დაყრდნობილი წამოცმის პოტენციალი იქნებოდა უფრო ეფექტური. ეს ვარაუდი შემოწმებულ იქნა კრასკელ-უოლისისა და წამოცმის მეთოდების გამოყენებით 73 მონომერულ ცილაზე და 104 მულტიმერული ცილის სუბერთეულზე. ნაჩვენებია, რომ ორივე მახასიათებელზე დაყრდნობილი პოტენციალის ეფექტურობა მნიშვნელოვნად აჭარბებს მხოლოდ x^0-ზე (ორიენტაციაზე) დაყრდნობილი პოტენციალის ეფექტურობას.

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MUTANT STRAIN OF *PENICILLIUM CANESCENS* WITH HIGH XYLANASE ACTIVITY

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Abstract

Penicillium canescens RTM-22 a mutant strain, which is characterized by increase of xylanase activity by 500% in comparison with the original strain, has been obtained against a background of cellulase zero activity. Optimum growth conditions have been selected and also features of xylanase have been studied. Preparation of xylanase obtained by different organic solvents has been applied in the technological process of delignification and significant results have been achieved in this direction. Particularly, preparation of xylanase has decreased lignin content by 16% in whitened cellulose and therefore index of whiteness of cellulose has been increased by 50%. It enables to exclude the stage of chlorination from the schedule of cellulose whitening, which is very important ecologically.

Key words: hemicellulose, xylanase, mutagenesis, a fermenter

Introduction

In paper industry, hemicellulose might be applied as a source of sugar capable for fermentation in order to obtain cellulose. In this case, zero level of cellulase activity is required during usage of xylanase preparation, since even the low cellulase activity decreases the quality of the soft mass (1). Strains with high hemicellulase activity are well-known in nature but generally, they are also characterized by high cellulase activity. Removal of cellulase by its inactivation or ion-exchange chromatography changes significantly the value of xylanase preparation [Dusterhoft et al., 1997, Prabhu et al., 1999, Elisashvili et al., 1999].

Materials and methods

The object of our investigation was *Penicillium canescens* RTM-20171, the strain is kept in the collection of microorganisms at the Institute of Biochemistry and Biotechnology, Academy of Sciences of Georgia and also some mutant strains obtained as a result of exposition of the original strain to ultraviolet rays. Cultures were grown in soluble universal medium both in Erlenmeyer flasks and fermenter of "New Branswich" (USA). The original strain was exposed according to the method elaborated at the Institute of Biochemistry and Biotechnology, Academy of Sciences of Georgia.

Xylanase activity was determined by the reagent of dinitrosalicylic acid [Bailey, 1990]. Endoglucanase activity was determined by the method of KMC. Common cellulase activity was defined towards Whatman filter paper N1 [Ghose, 1987].

Reducing sugars were determined by Somogyi'-Nelson's method [Nelson, 1944, Somogyi, 1952]. Amount of proteins was defined according to the method of Lowry- Bradford.

Results and discussion

The goal of our investigation was to obtain the strain with high hemicellulase activity, which would not display cellulase activity, also to receive fermentative preparation and elaborate the technological ways of its application.

Rich selective material has been obtained from micromycetes *Penicillium canescens* RTM-20171 via its exposure to physical mutagen, particularly to ultraviolet rays. About 80 cultures, which are characterized by both changeable and unchangeable morphological features, were isolated from single colonies. The exposure doze ranged from 3,5-1120 joule/sq.m. Study of xylanase and cellulase activities in the strains mentioned above showed that xylanase activity was increased from 50% to 500% in 24 cultures (30% of total amount), whereas cellulase activity was significantly decreased (Fig. 1). As it is seen from the results on the diagram the most interesting strain, hereinafter called as *Penicillium canescens* RTM-22, was nontoxic and nonpathogenic one.

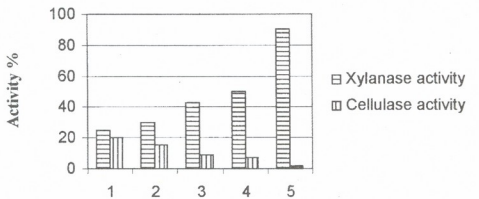

Fig. 1. Effect of irradiation dose (Joule/ sq.m) on xylanase and cellulase activities

1-Control; irradiation dose (J/sq.m): 2 - 3,3; 3 - 6,6; 4 - 60; 5 – 390

At the next stage of the work optimum condition of the strain growth was selected. It was found that maximum amount of xylanase was synthesized at 70 hours of deep cultivation, at 28°C. In order to optimize the medium, different carbohydrates and various waste products with rich content of carbohydrates were introduced to it. As it is shown from the results, addition of 1% of corncob increased xylanase activity by 50-60% (Fig. 2) and addition of 1% of vitamin-complex (biotin, riboflavin, thiamine, nicotinic acid, B_{12}, B_6) increased amount of the enzyme by 50%.

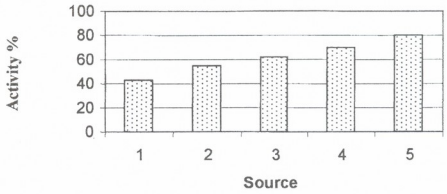

Fig.2. Effect of carbon (c) source on the activity of xylanase from *Penicillium canesces*

1-Control; 2- citrus west, 3- maize extract; 4- malt; 5- maize

During cultivation over 90% of the enzyme synthesized by fungus was transferred to culture liquid. Thus, filtrate of culture liquid was used to obtain technical preparation by applying the methods of protein precipitation with organic solvents – acetone (1:1) and alcohol (1:1,5).

Determination of the indices such as optimum temperature - 55°C and optimum pH 4,2-4,5 preceded to application of technical preparation in semi-industrial purpose. Study of thermal stability showed that the enzyme maintained its activity for an hour, at 55°C.

At present much importance is attached to usage of hemicellulose to improve paper quality, by degrading only xilan.

On the base of received data the results have been established as follows:

a) The obtained preparation of xylanase is distinguished by its significant capability for delignification.

b)Optimum conditions for the enzyme activity were chosen in unbleached cellulose (temperature - 45°C, pH 4,5, duration- 2 hours, amount of added xylanase -1%).

c)Under the conditions mentioned above xylanase decreased content of lignin by 15, 8% in unbleached cellulose and therefore the index of cellulose whiteness increased by 5%.

d) Treatment of cellulose by fermentative preparation enables to reduce scheme of whitening by two stages. Exclusion of the stage of chlorination is very significant for the ecological point of view.

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მაღალი ჭსილანაზური აქტივობის მქონე მუტანტური შტამის მიღება *Penicillium canescens*-იდან.

ჯობავა მ., გომართელი მ., ხოხაშვილი ი., ქუთათელაძე ლ., ზაქარიაშვილი ნ., დაუშვილი ლ., საბაშვილი ნ., აპლაკოვ ვ.

საქართველოს მეცნიერებათა აკადემიის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 22.03.2004)

რეზიუმე

მიღებულია მუტანტური შტამი ***Penicillium canescens*** **RTM-22,** რომელიც თითქმის ნულოვანი ცელულაზური აქტივობის ფონზე ხასიათდება 500%-ით გაზრდილი ქსილანაზური აქტივობით საწყის შტამთან შედარებით. შერჩეულია ზრდის ოპტიმალური პირობები. შესწავლილია ფერმენტ ქსილანაზის თვისებები. სხვადასხვა ორგანული გამხსნელებით მიღებული ქსილანაზის პრეპარატი გამოყენებულია ცელულოზის დელიგნიფიკაციის ტექნოლოგიურ პროცესში და ამ მიმართულებით მიღებულია მნიშვნელოვანი შედეგები. კერძოდ, ქსილანაზური პრეპარატი დაახლოებით 16%-ით ამცირებს გათეთრებულ ცელულოზაში ლიგნინის შემცველობას, რითაც 50%-ით ზრდის ცელულოზის გათეთრების ხარისხს. ეს საშუალებას გვაძლევს სქემაში გამოვრიცხოთ ქლორირების ეტაპი, რაც ეკოლოგიურად ძალზედ მნიშვნელოვანია.

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Botany

DISTRIBUTION PATTERN OF GEORGIA'S FOREST BRYOFLORA AS OBSERVED ON THE TRIALETI RANGE

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Abstract

Lists of the dominant and characteristic moss species distributed in the forest formations of the Trialeti range have been compiled (according to substrate type) in consequence of investigations of many years. The distribution range of the *Hylocomium* community characteristic to fir forests have been determined in various altitudinal belts (800-2000m a.s.l.). It was shown that insignificant fluctuation of micro-edaphic and micro-climatic conditions does not support the moss diversity. The specific distribution pattern of mosses occurring under the canopy depends on the ecology of separate species in certain micro-topographical conditions.

Key words: forest, moss, phytocoenosis, Trialeti range.

Introduction

Forests of Trialeti and their bryoflora are worth attention owing to the fact that they include almost all principal formations of the Georgia's forests.

A purpose of the study was to find out interconnections between the forest mosses and surrounding communities.

Scandinavian phytocoenologists considered moss communities in connection with the substrate yet in the XIX century. Later other approaches started to develop in Europe.

It is obvious that moss communities are not independent; they are affected by other communities and related to certain microclimatic conditions thus forming a typical structural element of forests [Ariskina, 1962; Shennikov, 1964; Chikovani, 1968]

Materials and Methods

In consequence of itinerary and semi-stationary studies of many years carried out using geobotanical and parceling methods, we have determined various floristic complexes referring to separate formations; we have also revealed the distribution patterns of dominant and characteristic species in connection with particular types of stands, grass cover, underlay, litter, soil, etc. (Tables 1 and 2).

Results And Discussion

In the forests of the Trialeti range the moss communities exhibit the dependence upon certain edificatory. The *Hylocomium* community, which is composed of the following species, is considered as an example: *Pleurozium schreberi, Rhytidiadelphus triquetrus, Hylocomium splendens, Eurhynchium* striatum and other dominant and characteristic species.

Table 1. Participation of dominant and subdominant moss species in separate plots of various forest formations.

#	Stand Composition	Total cover (%)	Moss Species							
			Dicranum scoparium	*Hylocomium splendens*	*Rhytidiadelphus triquetrus*	*Hypnum cupressiforme*	*Isothecium myurum*	*Rhytidium rugosum*	*Mnium cuspidatum*	*Pleurozium schreberi*
1	Pine	70-80	+	+	+	+	*	+	+	+
2	Fir-Pine	80-90	+	+	*	+	+	+		+
3	Beech-Pine	60	+	+	*	+	*			+
4	Beech	90	+	+	+	*	+			
5	Fir-Beech	80	*	*	*	*	+			
6	Hornbeam-Beech	50	*	*	*	+	*			
7	Fir-Hornbeam	50	*	*	*	*	*		+	*
8	Oak-Oriental hornbeam	90	*	*	+	*	*	+	+	

+ - dominant, * - subdominant

Table 2. Frequency of occurrence and abundance of the forest moss species distributed in the principal forest formations of the Trialeti range.

#	Moss Species	Formations						
		Fir	Pine	Beech	Oak	Oak-Oriental hornbeam	Rhododendron	Oriental hornbeam
1	*Plagiochilla asplenioides*	3/0		3/0			3/0	
2	*"Hylocomium* formation"	3/0	2/0	1/+		1/+	3/0	
3	*Tortula subulata*	1/+		3/θ	2/θ	3/θ		
4	*Dicranum scoparium*	2/θ	3/0	2/θ		1/θ		
5	*Leucodon immersus*			1/θ	3/0	2/0		3/0
6	*Anomodon viticulosus*			2/0	3/0	3/0		
7	*Bryum capillare*	2/0	2/0	2/0	2/0	2/0		
8	*Mnium cuspidatum*		2/θ	3/θ		1/θ		
9	*Neckera complanata*	1/θ	1/θ	2/0				
10	*Eurhynchium striatum*	3/0	1/+	1/+				
11	*Brachythecium rutabulum*	2/θ		3/0				
12	*Hypnum cupressiforme*		3/0		2/θ	1/θ		
13	*Rhytidium rugosum*		3/0		2/0	2/+		

Key: 3 – Abundant, 2 – Mean, 1 – Scanty.
0 – Frequent, θ – Mean, + - Single.

We have studied young as wall as old stands of fir formations at elevations between 800 and 2000m a.s.l. on slopes of different topography and aspect.

1500-2000m a.s.l. – Fir forests with patches of mosses. Three micro-communities have been distinguished in this formation:

1. Fir forests with the following mosses: *Pleurozium schreberi, Rhytidiadelphus triquetrus, Hylocomium splendens, Eurhynchium striatum*;
2. Fir forests with the following dominant mosses: *Mnium undulatum* and *Mnium affine;*
3. Rarefied forests with *Tortula ruralis, Dicranum scoparium* (mosses characteristic to open places) on sward, overgrazed grass cover.

1700-1800m a.s.l.

1. Typical *Hylocomium* formation: *Hylocomium splendens, Pleurozium schreberi, Rhytidiadelphus triquetrus*, etc.

800-1300m a.s.l.

1. *Eurhynchium striatum* (a species of the *Hylocomium* formation) together with *Festuca montana* occur most frequently in fir forests spread at the noted elevations. In upper tiers silver fir is admixed.
2. Plots on slopes inclined towards a river, where the following mesophilous mosses dominate: *Atrichum undulatum* and *Mnium rostratum*.

800m a.s.l. – Fir does not form typical boreal complexes in the lower belt; however, moist conditions have caused the development of the noted complex of mesophilous mosses.

The *Hylocomium* formation noted above is not characteristic to the beech formation; however, it occurs in certain communities. Particularly, beech forest with moss cover is rather rare in the Caucasus [Tumadjanov, 1934]. The following species should be considered adapted to beech forests to a certain extent: *Fissidens taxifolius, F. cristatus, Anomodon viticulosus, A. attenuatus, Isothecium myurum, Pterygynandrum filiforme, Brachythecium rutabulum, Mnium immarginatum*, etc.

Epiphitic species *Orthotrichium lyelii, O. fastigiatum, Neckera pennata* should be considered characteristic to the fir forests; the *Hylocomium* formation is unquestionably dominant and characteristic. The noted species dominate over the mosses in the community of *Rhododendron ponticum* and occur in beech and oak-hornbeam forests. *Atrichum undulatum* is adapted to the silver fir community to some extent. The following species dominate in pine forests: *Dicranum scoparium, Rhytidium rugosum, Hypnum cupressiforme, Tortula ruralis, Thuidium abietinum. Orthodicranum montanum* can be found in dry litter. According to the data reported by a number of authors [Tumadjanov, 1934; Hernandez-Garcia et al, 1999; Juravliova E. N., Ipatov V. S., 2003; Tolpisheva T. U. et al, 2003] beside fir forests, well-formed moss communities occur in those short-lived pine forests, which are then replaced by fir forests.

In oak, hornbeam, beech forests a group of epiphitic mosses is best formed, because these forests lack continuous moss cover owing to the fall of the leaves. *Leucodon immersus* (mainly in oak forests) and *Isothecium myurum* (in beech forests) predominate among epiphites; species of the genus *Anomodon* occur frequently in oak forests and so forth. The following representatives of the genera: *Brachythecium* and *Plagiothecium* predominate on fallen leaves in beech forests:

Brachythecium rutabulum, B. salebrosum, B. rivulare, B. starkei, Plagiothecium denticulatum, P. neglectum, P. elegans. The following species are distributed in oak forests: *Pyalisia polyantha, Campilium chrysophyllum, Leskeella nervosa, Amblystegium serpens, A. juratzkanum* and the following in hornbeam forests: *Tortula ruralis, Brym capillare.*

High mountain species (*Distichium capillaccum, Polytrichum alpinum*, etc.) accompany the group of formations of *Rhododendron* and subalpine meadows.

It appears that the development of every moss community is connected with other communities through environmental conditions. Homogenous environment existing under the canopy formed by an edificator encourages the expansion of moss communities. However, sometimes the participation of mosses in the succession becomes apparent somewhat later, as it can be concluded according to dominant species. A moss community occurring under the canopy retains its principal characteristics even if the composition of a stand varies remarkably [Dolukhanov, 1958].

Thus, we have concluded that local micro-edaphic and micro-climatic conditions wit insignificant fluctuations does not support moss diversity. A specific pattern of the distribution of mosses occurring under the canopy depends on the ecology of separate species, which form different mosaic patterns on certain micro-topography.

We have observed a disturbed pattern of the moss adaptation related to the succession of formations. It must be due to rather late alteration of moss communities.

Succession of pine, fir and beech, replacement of pine forests by fir and fir and beech ones, which on the Trialeti range is referred to the late Holocene can be seen in the present spectrum of the broad-leaved forests spread in this region. This is additionally proved by the distribution pattern of the moss cover in the noted formations, as it was determined during our research. Studies of the structure of the present plant cover and succession of communities provide valuable data for the determination of the recent history of the plant cover.

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საქართველოს ტყეების ბრიოფლორის გავრცელების კანონზომიერება თრიალეთის ქედის მაგალითზე

ჩიქოვანი ნ.

საქართველოს მეცნიერებათა აკადემიის ნ. კეცხოველის სახ. ბოტანიკის ინსტიტუტი

(მიღებულია 14.04.2004)

რეზიუმე

დადგენილია თრიალეთის ქედის ტყის ფორმაციებში გავრცელებული ხავსების (სუბსტრატის მიხედვით) და გაბატონებული და დამახასიათებელი სახეობების სია. გამოვლენილია ნაძვნარი ტყეებისათვის დამახასიათებელი „ჰილოკომიუმის" სინუზიის არეალი სიმაღლის სარტყელების მიხედვით (800-2000მ ზ.დ.). გამოვლენილია, რომ მიკროუდაფური და მიკროკლიმატური პირობები მცირე რყევადობით ვერ განაპირობებს ბრიოფლორის მრავალფეროვნებას. ტყის საფარქვეშ შექმნილი სპეციფიკური ხავსების მოზაიკა დამოკიდებულია ცალკეულ სახეობათა ეკოლოგიაზე მიკრორელიეფში.

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Cytology

THE PARTICIPATION OF SUBVENTRICULAR CELLS OF DORSO-LATERAL WALL OF LATERAL VENTRICLE IN THE POSTNATAL DEVELOPMENT OF THE WHITE MICE CEREBRAL CORTEX

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Abstract

Proliferation, migration and differentiation of stem subventricular cells in dorso-lateral wall of lateral ventricle, Glial and neuroblastic cells proliferation in motor cortex and subventricular cells postmitotic derivatives migration and definite differentiation was studied at the early stages of postnatal development of white mice (from the 1st up to the 30th day after birth) by impulse and prolonged autoradiography methods with the application of ^{3}H-thymidine. The results have shown that with the aging of mice the proliferation activity notably decreases and already by the 30th day the matrix is almost completely realized. A number of subventricular cells migrates to the motor cortex and white matter. Consequently, motor cortex enrichment by subventricular cells mitotic derivatives is increased. The bulk of migrated feebly labeled cells is comprised of glial population, although small neurons are also presented low amount.

Key words: subventricular zone, cortex, proliferation, migration, identification, impulse and prolonged autoradiography

Introduction

It has been established at present that a certain equilibrium level of indifferent stem cells is preserved in the cerebrum of even adult mammals in postnatal period, and by means of proliferation, migration and consequent differentiation, the cortex is enriched with new populations of nerve and glial cells [Snyder, 1994, Gage, 2000, Luskin, 2001].

Although, yet in 70-80-ies the scientists from the Morpho-Physiology Laboratory have established [Mepisashvili, 1970/71, 1982, Kalandarishvili, 1989], that at early stages of postnatal ontogenesis of animals indifferent subventricular cells are preserved at a different extent both in cranial, central and caudal sections of dorso-lateral wall of lateral ventricle and migrate to the corresponding areas of hemisphere cortex. With the age, these processes cease gradually. An extent of subventricular cell conservation degree in different mammals is dissimilar and carries species-related features. These differences are particularly conspicuous when comparing mature-born animals (guinea pigs) with immature-born ones (mice, rats, rabbits, cats, dogs and humans). Furthermore, we have observed a certain relationship from the phylogenetic point of view in immature-born animals: the higher the organisation of an animal is, the later the functioning of its

germinative zone is completed, and respectively the formation of the hemisphere cortex [Mepisashvili, 1982]. It has been established that the rate of the cell equilibrium state in each section of the subventricular zone is directly related with the functional activity of the hemisphere cortex [Kalandarishvili, 1989]. A possibility of subventricular cells participation in reparation of cortical lesions in early postnatal period was also demonstrated [Mepisashvili et al., 1968].

The objective of this investigation is to study the realization dynamics of the subventricular cells of the lateral ventricle dorso-lateral wall over the period of early postnatal development of cerebrum in white mice.

Materials and Methods

30 white mice of 1 – 30 days after birth was the object of the investigation. The isolated brains were fixed in Carnoy liquid and, after proper dehydration, their 7 μm paraffin sections were stained with hematoxylin-eosine according, with cresyl-violet and according to combined method with ferrous hematoxylin and further staining with cresyl-violet.

In order to detect proliferation processes, quantitative registration of cells synthesising DNA and dividing mitotically was conducted in 3 sections of lateral ventricle dorso-lateral walls: cranial, central, caudal, and also in one of the areas of neocortex (motor cortex), by method of impulse, short-term. In these experiments ^{3}H-thymidine (mol. activity is 2,368) in dose of 10 μCi/g was introduced intraperitoneally at a single injection 1 hour prior to sacrifice of 1-30 day animals. For revealing of migration processes and for definite localisation of ^{3}H-thymidine labelled postmitotic derivatives of matrix cells, as well as for their identification the method of prolonged, cumulative autoradiography was applied. ^{3}H-thymidine in a dose of 5 μCi/g was introduced to 3-day mice 3 times a day at 3 hour intervals.

Animals were sacrificed 7 hours after the first injection, on the 7th day after birth and on the 30th day after birth. a) the number of labelled cells migrating in that period (from the 3rd to the 30th day) from subventricular zone to motor cortex; b) identification of preliminarily labelled subventricular derivatives that definitely localized in the motor cortex differentiated into nerve and glial elements.

Results and Discussion

The subventricular zone of dorso-lateral wall of the lateral ventricle in newborn mice is well expressed. The cells in this zone are heterogeneous in their composition and are at different stages of differentiation. The major portion of the cells is comprised of indifferent stem matrix cells (Fig. 1) that are most richly represented in the cranial section. The results of calculation of DNA-synthesising and mitotically dividing cells have shown that the labeling index (LI) in new-born mice and the mitotic activity index (MAI) in all 3 studied sections were characterized by relatively high values. With the age their number notably decreases and already in 30-day mice the proliferating cells are found in insignificant amount. Along with this, both the LI and MAI in cranial section in all age groups of animals are significantly higher than in central and caudal sections (Fig. 2).

The data of prolonged cumulative autoradiography have shown that in starting 3-day mice (1st group) the majority of DNA-synthesising cells in subventricular zone contained intensive label (Table 1).

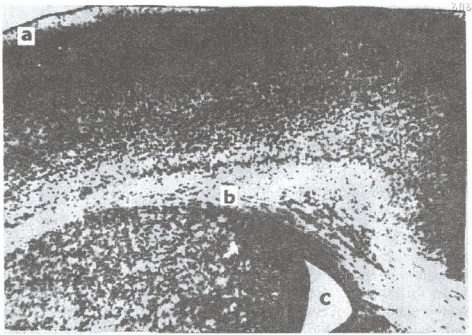

Fig. 1. General view of the front part of the left hemisphere motor cortex (a) with cranial part of dorso-lateral wall (b) of lateral ventricle(c) in 1-day mice. Stained with cresyl-violet Magnification obj. 4, ocu. 7.

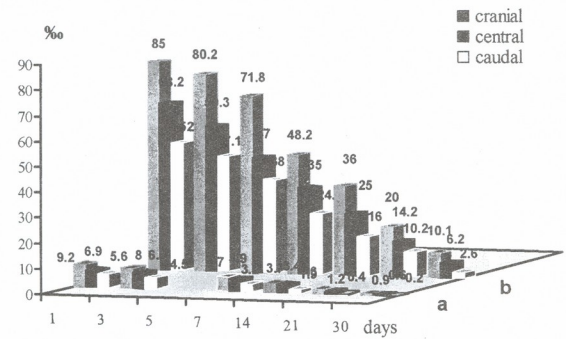

Fig. 2. Mitotic activity (a) and the number of DNA syinthesing subventrical cells (b) in mice of various age abscises axis-days after birth; ordinate axis-the number of mitotic and DNA synthesising cells

Table 1. The number(%) of labeled cells in subventricular zone in 3 parts of dorso-lateral walls of lateral ventricles in 3-day mice at various periods after triple injection of ^{3}H-thymidine

Sacrifice periods after ^{3}H-thymidine injection	Parts of subventricular zone					
	Cells with non-feeble label			Cells with feeble label		
	Cranial	Central	Caudal	Cranial	Central	Caudal
7 hours	119	98	70	-	-	-
24 hours	-	-	-	150	123	90
4 days	-	-	-	78	60	48
27 days	-	-	-	9	4	2

Table 2. The number of labeled cells in motor cortex at various dates after triple injection of ^{3}H-thymidine in 3-day mice

Age of mice (days)	Total number of labeled cells	Cells with intense label	Cells with weak label
3	48	41	(7)
7	152	13	139
30	204	5	199

Table 3. The number of labeled cells migrated from the lateral ventricle subventricular zone to the motor cortex.

Migration dates	The number of labeled cells
From the 3-rd to the 7-th day	83
From the 7-th to the 30-th day	44
From the 3-rd to the 30-th day	127

In 4-day mice (after triple injection of ^{3}H-thymidine), a small fraction of labelled cells evidently has managed to migrate. However, the major part of labeled cells, though divided, is still localized in subventricular zone. Therefore, this group of animals shows the highest amount of cells with feebly linked label.

In 7-day mice, the amount of labelled cells notably decreases, since in that period most of the cells migrated to the cortex and white matter that can be easily judged by the following: 1) a marked dilution of subventricular zone, 2) clearly visible traces of migrating feebly labeled cells and 3) sharp decrease of LI (Table 1).

In 30-day mice, the stem cells in subventricular zone are almost completely realized as a result of their migration to the cerebral cortex. Only their small clusters are present along the ependymal lining. The amount of feebly labeled cells is insignificant.

The results of studies on the local histogenesis dynamics in motor cortex cell composition with respect to the amount of mitotically dividing and DNA-synthesising neuroblastic and glial cells have shown that there is a postnatally preserved minor supply of neuroblastic-type cells still retaining their histoblastic potential. However, their number is insignificant compared with the glial population. With the age the proliferation processes cease abruptly (Fig. 3).

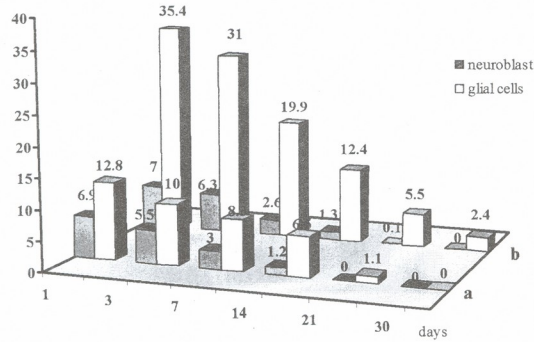

Fig. 3. Average number of mitotic (a) and labeled (b) neuroblastic and glial cells of brain motor cortex in mice of various age. Abscises axis-days after birth; ordinate axis-the number of mitotic and labeled cells

The results of prolonged autoradiography have shown that after triple injection of ^{3}H-thymidine in 3-day mice an initial total number of labeled cells in a local section of motor cortex is 48 in average (Table 2). Overwhelming majority of these (41) were intensely labeled cells, and only a minor amount (7) comprised a population of weakly labeled cells. It should be noted that local, initially cumulatively labeled cells in 3-day mice were mainly detected in upper layers of motor cortex and they are similar to the impulse-labeled population. Because of the low level of differentiation, it is quite difficult to identify them distinctly at this age.

In 7-day mice, the total number of labeled cells comprised in average 152 per section, i.e. it increased almost 3,2 times compared with the number in 3-day mice. Among these cells, 13 were intensely labeled, while 139 cells were feebly labeled. By this period the number of locally formed cells in the cortex was 56.

In 30-day mice the total number of labeled cells is 204, i.e. over 5 times more as compared with 3-day mice. The major share of the labeled cells at that is comprised of feebly labeled ones – 199, while the number of intensely labeled cells has decreased to 5. So, in the period between the 7th and the 30th day the motor cortex is enriched with less amount of cells at the expense of local

proliferation – 16, and this confirms the data of short-term impulse autoradiography. Thus, the significant growth of the number of feebly labeled cells observed in the motor cortex of the 30-day mice is mainly due to migration from subventricular layer of post-mitotic derivatives of matrix cells.

On the basis of above-mentioned data, we have calculated the number of labeled cells that migrated from subventricular zone to motor cortex in 7-day and 30-day mice. In 7-day mice the number of feebly labeled cells migrating from subventricular zone to motor cortex was 83 (Tab. 3).

The number of feebly labeled cells migrating from subventricular zone to motor cortex between the 3rd and the 30th day was 127. So, between the 7th and the 30th day only 44 cells have migrated from subventricular zone to motor cortex.

Thus, the method of long-term prolonged cumulative autoradiography has enabled us to supplement significantly the data of short-term impulse autoradiography and to reveal certain relationship in redistribution of labeled cells from subventricular zone to cerebral cortex: as the number of labeled cells in subventricular zone decreases, the number of labeled post-mitotic derivatives enriching the cerebral cortex is notably increasing.

The method of prolonged autoradiography has enabled us to determine the type of post-mitotic derivatives of subventricular cells that within the studied period between the 3rd and the 30th day migrate to cortex, get their definite location and appreciably differentiate.

As has been shown by the results of this investigation, the major part of feebly labeled cells is comprised of neuroglial population, though in low amount there are also nerve cells, mainly small-size neurons.

Along with the data on the number of feebly labeled nerve cells enriching the cerebral cortex, we have also presented the data for glial population. The prolonged autoradiography data have shown that in the period of early postnatal ontogenesis, at the expense of matrix, the cerebral cortex is enriched with much higher numbers of glial cells (101), than of the nerve ones (26) that is well explained by ceasing of prolifeation processes linked with production of the nerve elements. Sharp increase of glial population in the cortex of postnatally developing animals, as compared with their local proliferaion trend, obviously should be explained by the migration of newly formed derivatives of neuroglia from subventricular zone.

Thus, long-term prolongated autoradiography enabled us to establish further fate of post-mitotic derivatives of subventricular cells and led to a conclusion that postnatally the dorso-lateral walls of lateral ventricles in mice do not lose their histoblastic potential and continue enrichment of the cortex with new populations of both glial and small neurons.

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გვერდითი პარკუჭების დორსოლატერალური კედლის სუბვენტრიკულური უჯრედების მორანჟილეობა თეთრი თაგვების თავის ტვინის ქერქის პოსტნატალურ განვითარებაში

კალანდარიშვილი ე. თაქთაქიშვილი ა. ბუკია რ. გელაშვილი ნ.
დავითაშვილი მ. მაჭავალაძე ნ.

მორფო-ფიზიოლოგიის საპრობლემო ლაბორატორია, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 04.05.2004)

რეზიუმე

პოსტნატალური განვითარების ადრეულ ეტაპზე (1-30 დღე) შესწავლილია თავის ტვინის გვერდითი პარკუჭების დორსოლატერალური კედლის ჰერმინატული (მატრიქსული) და ქერქის მამოძრავებელი მიდამოს ჰისტობლასტური პოტენციის გლიური და ნეირობლასტური ტიპის უჯრედების პროლიფერაცია; აგრეთვე სუბვენტრიკულური უჯრედების პოსტმიტოზური დერივატების მიგრაცია და საბოლოო დიფერენცირება, იმპულსური და პროლონგირებული ავტორადიოგრაფიის მეთოდებით ^3H -თიმიდინის გამოყენებით. ნაჩვენებია, რომ აღნიშნული უჯრედების პროლიფერაცია ასაკობრივად მცირდება. პოსტემბრიონალური განვითარების 30-ე დღისათვის მატრიქსი თითქმის რეალიზებულია, გვხდება მატრიქსული უჯრედები ცალკეული გროვების სახით. ხოლო სუბვენტრიკულური უჯრედების ძირითადი მასა მიგრირებს ტვინის თეთრ ნივთიერებასა და ქერქში. შესაბამისად, მნიშვნელოვნად იზრდება მათი პოსტმიტოზური დერივატებით ქერქის შევსება. მიგრირებული უჯრედები ძირითადად წარმოდგენილია გლიური ჰოპულაციებით, თუმცა მცირე რაოდენობით აღინიშნება ნეირონებიც.

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Cytology

THE INFLUENCE OF HEPAR COMPOSITUM ON REGENERATIVE GROWTH OF WHITE RAT HEPATOCYTES AFTER PARTIAL HEPATECTOMY IN CONDITIONS OF CHOLESTASIS

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Abstract

The influence of antihomotoxic drug Hepar compositum on adult white rat hepatocytes regeneration in condition of cholestasis has been studied. The influence of drug has been estimated based on the changes of transcriptional activity of hepatocyte nuclei and morphologic features of liver tissue. It has been demonstrated that after intraperitoneal injections of Hepar compositum the transcriptional activity of hepatocyte nuclei increases at the 6th hour after partial hepatectomy compared to control. The morphologic studies of liver tissue revealed dystrophic and necrotic changes. It was shown that antihomotoxic drug Hepar compositum has ability to stimulate transcriptional activity in condition of cholestasis while the morphologic evidences of regenerative growth are absent.

Key words: Hepar Compositum, partial hepatectomy, cholestasis, transcription, early response genes.

Introduction

The biosynthesis, transportation and metabolism of bile acids are necessary for normal liver functioning. The failure of their metabolism is linked with processes in liver which in turn influence the metabolism of bile acids. The partial liver resection made during surgical interventions, which include icterus of different etiology, has different influence on regeneration of remained tissue [Hardy, 1998].

Also it is known that cholestasis induces apoptosis and degenerative changes in liver tissue [Bird 2002]. However it has not been studied in details the processes which take place at early stage of regenerative growth during cholestasis. It is estimated that liver regeneration after partial liver resection during cholestasis is decreased [Ueda, 2002]. The regenerative growth also is diminished in conditions of external bile duct drainage [Cherrqui, 2000].

The study of changes of transcriptional activity of hepatocyte nuclei is very common today. American scientists studied the relation of early response genes to extrahepatic cholestasis.

They revealed that despite the increase of some basic transcriptional factors production no influence has been noted on this group of genes [Larkin, 2003].

The aim of our investigation was to reveal the influence of antihomotoxic drug Hepar compositum on adult white rat hepatocytes regeneration in condition of cholestasis.

Materials and Methods

40 adult white rats were used (100-140 g). Animals were divided into three groups. In first group cholestasis was caused by the common bile duct reduction and partial hepatectomy was performed; in animals of the second group 2µl/kg of Hepar compositum was injected and the common bile duct reduction and partial hepatectomy were performed; in the third group the common bile duct reduction and partial hepatectomy were performed and at the 5th hour 2µl/kg of Hepar compositum was injected. The influence of Hepar compositum on transcriptional activity of hepatocyte nuclei was estimated at the 6th hour after partial hepatectomy by method described earlier [Dzidziguri, 1997]. The material for histological studies was taken at the 4th day of bile duct ligation.

Results and Discussions

We revealed that in animals of the second group the injection of 2µl/kg of Hepar compositum stimulated the expression of genes (Fig. 1.). The RNA synthesis was also stimulated in the animals of the third group (Fig. 1.). The fact that Hepar compositum induced the increase of transcriptional activity in conditions of cholestasis points to the high stimulatory ability of this drug.

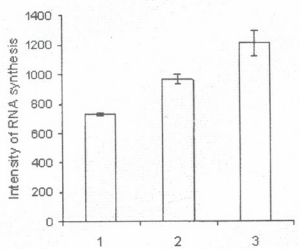

Fig. 1. Influence of hepar compositum on the hepatocytes stimulated for proliferation in conditions of hormonal disbalance

1. cholestasis and hepatectomy (6th hour)
2. Hepar compositum (1 hour) + cholestasis and hepatectomy (6th hour)
3. cholestasis and hepatectomy + Hepar compositum at the 5th hour

The histological examination of liver tissue revealed: dilatation of bile ductules, dilatation of central and portal veins, lymphocytic and histiocytic infiltration in triadic region with enlarged nuclei, reaction of Kuppfer's cells in center of lobule as well as in periportal zones (Fig. 2). The bigger magnification reveals the presence of the edematic hepatocytes with homogenized

cytoplasm and massive picnosis of nuclei around the central vein (fig. 3,4) in the center of lobule. In liver parenchyme the portal fields with massive lympho-hystiotic infiltration are visualized, in the first zone of acinusis the homogenization of cytoplasm is seen, also the clear dilatation of bile ductuli and the fields of proliferation of epithelium in bile ductuli is evident (fig. 5).

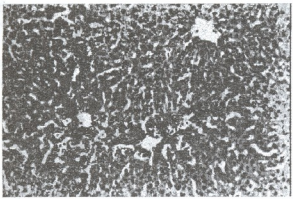

Fig. 2. The dilatation of bile ductules, dilatation of central and portal veins, lymphocytic and histiocytic infiltration in triadic region

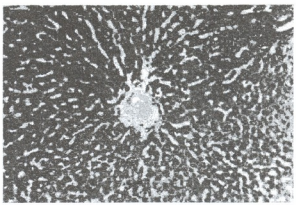

Fig. 3. The presence of the edematic hepatocytes with homogenized cytoplasm and massive picnosis of nuclei around the central vein

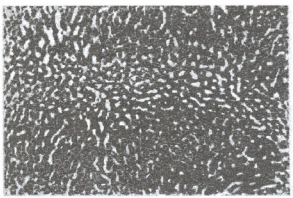

Fig. 4. The portal fields with massive lympho-hystiotic infiltration are visualized, in the first zone of acinusis the homogenization of cytoplasm is seen

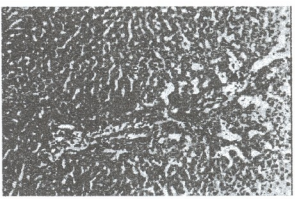

Fig. 5. The clear dilatation of bile ductuli and the fields of proliferation of epithelium in bile ductuli is evident

Our results make us to conclude that antihomotoxic drug Hepar compositum on early stage of regenerative growth has stimulatory effect on transcriptional activity of hepatocytes. This effect may be due to the activation of early response genes. But regenerative processes are not revealed during histologic examination - on the contrary, the degenerative-necrotic changes of liver tissue are clearly visualized. It's evident that prolonged morphologic evaluation of drug effect is necessary to make the final estimation of regenerative abilities of Hepar compositum.

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ანტიჰომოტოქსიკური პრეპარატ Hepar compositum-ის ზემოქმედება თეთრი ვირთაგვას ჰეპატოციტების რეგენერაციაზე ქოლესტაზის პირობებში

ლუარსაბიშვილი ვლ.[1], ცაგარელი ზ.[1], მეგრელიშვილი გ. [3], მაისურაძე ნ.[2]

[1]ა. ნათიშვილის სახელობის ექსპერიმენტული მორფოლოგიის ინსტიტუტი.
[2]ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი
[3]კ. ერისთავის სახელობის ექსპერიმენტული და კლინიკური ქირურგიის ეროვნული ცენტრი

(მიღებულია 26.04.2004)

რეზიუმე

შესწავლილია ანტიჰომოტოქსიკური პრეპარატ Hepar Compositum-ის ზემოქმედება თეთრი ზრდასრული ვირთაგვას ჰეპატოციტების რეგენერაციაზე ქოლესტაზის პირობებში. პრეპარატის ზეგავლენა შეფასებულია ჰეპატოციტების ბირთვების ტრანსკრიფციული აქტივობისა და ღვიძლის ქსოვილში მიმდინარე მორფოლოგიური ცვლილებების შესწავლის მიხედვით. ნაჩვენებია, რომ Hepar Compositum-ის ინტრაპერიტონიალურად შეყვანის შედეგად ჰეპატოციტების ბირთვების ტრანსკრიპციული აქტივობა კონტროლთან შედარებით იზრდება. მორფოლოგიური შესწავლის დროს კი ღვიძლის ქსოვილში დისტროფიულ-ნეკროზული ცვლილებები აღინიშნება. მიღებული შედეგებიდან გამომდინარეობს, რომ ანტიჰომოტოქსიკურ პრეპარატ Hepar Compositum-ს აქვს ტრანსკრიფციული აქტივობის სტიმულაციის უნარი, თუმცა ქსოვილის მორფოლოგიური შესწავლის დროს რეგენერაციისათვის დამახასიათებელი ცვლილებების განვითარება არ ხდება.

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Ecology

ANOPHELES MACULIPENNIS PHENOLOGY IN SOME HYPERMALARIOGENIC ZONE REGIONS OF GEORGIA

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Abstract

The observations of *Anopheles maculipennis* phenology were carried out in some hypermalariogenic zone regions of west and east Georgia. The seasonal biological materials for the main transmitter of malaria are analyzed in accordance with the mean daily temperature of air in separate territories. The phenological data have an epidemiologic importance in the transmission of malaria.

Key words: *Anopheles maculipennis*, parasitism, polyphagia, diapause, dissociation, honotrophic cycle, biological control.

Introduction

From the mosquitoes united in the genus malaria, *An. maculipennis* is especially widespread in Georgia. It is an active attacker, temporarily free ectoparasite, thermophilar ednophilar species that is harmful for human health not only as an ectoparasite but it also represents the transmitter of various pathogenic microorganisms including all four types of plasmodium that cause malaria - dangerous parasitic disease of human [Zvantsov, et al., 2003].

The study on Anopheles maculipennis phenology according to the malariogenic zones is of scientific and practical importance [Artemeev, et al., 1988].

A pre-imago phases, the beginning and finishing preventive activities against imago and the planning of antimalarial measures, are closely connected with the start and end dates of the transmitter's seasonal activity [Artemeev, et al., 1988].

The goal of research was to define the phenology of *Anopheles maculipennis* i.e. the basic moments of seasonal biology and life expectancy within the several hypermalariogenic zone regions. In Georgia since 1970s has not been carried out any scientific investigation on the phenology of *Anopheles maculipennis*, after 30-35 years we were interested in types of active life changes of the transmitter, especially as a result of sharp fluctuations of climatic factors on the base of global warming [Markovich, 2003].

Material and Methods.

Objects for the phenologic observations were selected from territories of the following regions: Gardabani, surroundings of Tbilisi (Soganlugi, village Digomi), Kaspi, Samtredia and Kobuleti. The observation for each object was carried out within 3 control points, where the

collection of material was collected twice per month according to the pre-imago and imago phases (worm, larva). Air and water temperatures were measured in each case of experiment. Asmani's psycho-meter was used in order to measure air temperature. A qualitative and quantitative analysis of collected material was performed in the laboratory. To estimate the collected material in accordance with separate object the known methodology was used [Zvantsov, et al., 2003].

Results and Discussion.

The duration of transmitter's seasonal activity is depended on the local climate. As the climatic conditions are vastly variable in Georgia, the phenologic dates of the mosquito according to the malariogenic zones are revealed in different time [Gugushvili, Sekhniashvili, 1999]. On the basis of our studies the following issues have been stated on the phenology of Anopheles maculipennis:

1. Flying away of females from wintering period, beginning of blood-sucking and honoactivity.
2. The development of first generation.
3. The development of last generation.
4. The fattening of females, the completion of nutrition and honoactivity.
5. A link between flora and phenologic events.

Mass activity of the transmitters within the hypermalariogenic zone appears in the third decade of February and the first decade of March in relation with the average 9-10°C daily temperature of air. In the districts of Tbilisi and in Gardabani the mentioned date is approximately the first decade of March, while in case of western Georgia (Samtredia, Kobuleti) the appearing of honoactive females in some years is noticed in the third decade of February.

The date of flying of first generation transmitters is also connected to the mean daily temperature of air. In the hypermalariogenic zone of west and east Georgia this process is registered during the third decade of April and the first decade of may, at 16,3-23,2°C. The dates of the fattening and honoactive processes taking place in females are determined - 12,6°C-15,5°C that corresponds to the second decade of October and the first decade of November. The pre-imago phases (worm, larva) are finally registered in biotopes in the third decade of October and the first decade of November at temperature - 12,9°C-17,4°C. To identify an epidemiological importance of the transmitter it is necessary to define the starting and finishing dates for the first sporogonic cycle in accordance with vertical zone. The average daily temperature of air comparatively accelerates or slows down the rate of the process. The development of the first sporogonic cycle within the female body appears when the mean daily temperature of air in the environment reaches 16°C, it correlates with the flying away date of the first generation of the transmitter. According to the observation, the females which passed the wintering period have no importance in the transmission process of malaria, because after flying they quickly die. Only the population of the first generation has an epidemiologic importance. It has been found that on the base of theoretical calculations the females affected with sporozoites in the hypermalariogenic zone of western Georgia might appear in the second, third decades of June. They may participate in the transmission process of malaria.

Data obtained from the studies on *Anopheles maculipennis* phenology enables us to conclude: the active life period of the transmitter in the hypermalariogenic zone of Georgia, begins from the second half of February and lasts up to the end of October or the first decade of November - for 8-8,5 months (table 1). The control on pre-imago phases should be started from the second or third decades of April up to the second half of October in relation with defining of disruption date of mass blood-sucking activity in females. The harmless control method on pre-

imago phases represents a broad spread of fish Gambusia within the anophelogenic water containers immediately from the beginning of mass flying away process of the first generation of *Anopheles maculipennis*. The mentioned date is in I-II decades of May in the hypermalariogenic zone of west and east Georgia. Thus, the seasonal phenomena of *Anopheles maculipennis* phenology depend on the macro- and microclimate of the environment. In the hypermalariogenic zone the optimal ecological conditions determine high quantity of the population that together with other factors sharply prolong the season of malaria transmission.

Table 1. Studies of *Anopheles maculipennis* phenology in some hypermalariogenic zone of Georgian regions

№	region	Appearing of honoactive females	Air temperature C^0	Development of first generation	Air temperature C^0	Development of last generation	Air temperature C^0
1.	Samtredia	20 II – 28 II	15,5 – 17,2	25 IV- 3 V	17,8 – 18,5	14 X – 20 X	12,8 – 16,0
2.	Kobuleti	15 II – 30 II	11,8 – 13, 5	30 IV- 2 V	16,8 – 19,0	22 X – 28 X	13,4 – 15,8
3.	Kaspi	17 II – 21 II	10,5 – 12,8	30 IV- 3V	17,5 – 18,0	15 X – 21 X	14,0 – 16,9
4.	Gardabani	10 II – 27 II	12,6 – 13,8	25 IV-5 V	18,0 – 19,9	17 X–18 X	11,5 – 15,0

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Anopheles maculipennis-ის ფენოლოგიის ძირითადი საკითხების ეკოლოგიური მიმოხილვა საქართველოს ჰიპერმალარიოგენური ზონის ზოგიერთ რაიონში

დეველაშვილი ბ.

ს. ვირსალაძის სახ. სამედიცინო პარაზიტოლოგიისა და ტროპიკული მედიცინის სამეცნიერო-კვლევით ინსტიტუტი.

(მიღებულია 12.04.2004)

რეზიუმე

მოცემულია მალარიის ძირითადი გადამტანის *An. maculipennis*-ის ფენოლოგიის შესწავლის შედეგები საქართველოს ჰიპერმალარიოგენური ზონის ზოგიერთ რაიონში. შესწავლილია მდედრების მეზამთრეობიდან გამოფრენა, სისხლწოვისა და ჰონოაქტიური ცხოვრების დაწყება, პირველი გენერაციის განვითარება, დაცხიმიანება, დიაპაუზა, ჰონოაქტივობის დასრულება, უკანასკნელი გენერაციის განვითარება. განსაზღვრულია სპოროგონიის პირველი და უკანასკნელი ციკლების დაწყებისა და დამთავრების ვადები; მალარიის ტრანსმისიის სეზონის ხანგრძლივობა, მალარიის ძირითადი გადამტანის *An. maculipennis*-ის აქტიური ცხოვრების დაწყებისა და დამთავრების ვადები, რომელიც 8-8,5 თვეს უდრის. კვლევის შედეგები საფუძვლად დაედება An. maculipennis-ის წინააღმდეგ პრევენციული ღონისძიებების ჩატარებას.

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MONITORING OF BENTHOS FAUNA OF GEORGIAN REGION OF THE BLACK SEA SHELF

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Abstract

Study of south-east region of the Black Sea Shelf has shown that the biocenosis of Chamellia (*Chamelea gallina*) with sand, muddy sand and mud sediment prevails. Benthos fauna is represented with 92 species of invertebrate animals belonging to several large taxons: 2 species from Sarcodines, 1 from - spongiforms, Turbellaria, Nematodes, Olygochaeta, Phoronidea and Echinodermata, 24 from Polychaeta, 21 Crustacea and 39 mollusks, among them 19 gastropodes and 20 – bivalved mollusks. The dominant and subdominant species in this particular area are: Lanellibranchiata s. Bivalvia (*Ch.gallina, C.cornea, L.mediterranium, B.triangular*), Echinodermata (*A. Stepanovi*), Gastropoda (*C.donovani*), Crustacea (*B.improvisus*). In Georgia *C.cornea* was registered for the first time.

Key words: *Cunearca cornea, Olygochaeta Crustacea, Gastropoda, Lamillibranchiata, Echinodermata.*

Introduction

The south-east region of the Black Sea Shelf is distinguished with its rich and diverse benthos fauna. The fishes of this region mainly feed on benthos organisms and thus determining of the species is one of the most prior tasks. We were especially concerned with the section between Batumi-Kobuleti along the Black Sea Shelf, where the most part of the benthos fauna is gathered and which has not been systematically described in Georgia for the last 20 years.

We have held short term monitoring of the sea front along Batumi-Kobuleti section in 2000-2002.

Material and methods

The monitoring of the benthos along the Black Sea Coast in Ajara region in Georgia took place in 2000-2002. The material has been obtained with a drag and the bottom scoop in the 16 points of 3-30 meters isobaths. There have been 106 benthos samples collected and processed on the whole. The research stations have been separated approximately at 0.5-3 miles from each other.

In order to perfect the biodiversity of the benthos, 16 dredging indices of the fishing have been used.

Benthos samples were withdrawn and fixed with proper fixatives. Their systematization was done in the laboratory according to the known method.

Results

In the faunistic complex of the studied region of the Black Sea Shelf the mollusks are distinguished with the species diversity. In Georgia *C.cornea* is mentioned for the first time, which is the object of monitoring. According to the scientific data young samples of this mollusk were observed in 1968 in the shelf of Caucasus. In 1982 *Anadera sp.* was fond in the Varna Bay, but it's taxonomy was not exactly defined [Marinov, 1990]. Later it was defined by Romanian hydrobiologist Gomoius as *Schaphara ineaquovalvis* [Gomoius, 1997]. *Cunearca cornea* was found for the first time by Starobogatov in 1978-1979 during the study of the benthos fauna of the Georgian Black Sea seafront and in the rivers Chorokhi and Belaia on 5-20 m. isobaths. This mollusk was sized 1-2,5 cm., while the size of this species caught on the fishing plain "Sigzbi" is significantly more – 6-8 cm.

The self acclimatized opportunistic species, filtrator – *Cunearca* is widely spread. This species has massive shell with ability of hermetic closing and in the case of oxygen deficiency in the sea bottom they can endure hypoxia. The other registered by us bivalved mollusks have not this capability, what is the reason of getting this species among the dominants.

The table represents the taxonomical accessories of species and their distribution to different catching points. The table shows that the number of dominants and subdominants is small and majority of species belongs to accidents. So the main attention of the monitoring is paid to the cenoses-produced species and dominants which enables us to have some notion about cenoses of benthos organisms. The possible changes of species in cenoses indicates the necessity of the monitoring.

Table 1. The benthos diversity of the Black Sea coast of Georgia (Ajara region)

Taxonomy	Organisms obtained at different points															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Protozoa-Sarcodina																
1-Streblus becarii																+
2. Quinguelosulina pseudoseminula																+
Porifera-Porifera																
1. Sycon ciliatum																+
Plathelminthes- Turbelaria																
1. Stylochus pilidium																+
Nemathelminthes Nematoda																
1. Nematoda sp.	+							+								
Annelidae Polychaeta																
1. Aricidea cerrutii	+				+			+						+	+	
2. Ancristrosullis tentaculata	+	+														
3. Capitellides qiardi								+								
4. Eteone picta								+	+							+

5. Glycera tridactula								+				+				
6. Heteromastus filiformis				+	+		+									
7. Micronephthys stauneri	+	+		+			+	+		+				+		
8. Microspio mecmikowianus	+															
9. Melinna palmata	+	+	+	+		+	+	+			+			+		
10. Magelona rozea														+		
11. Magelona papilicomis		+														
12. Nereis succinea				+				+								
13- Nereis zonata				+												
14. Nereis fucata																+
15. Nereis divesicolor																+
16. Nereis longissima																+
17. Nephthys cirrosa	+	+											+	+	+	
18. Nephthys hombergii	+	+	+	+			+	+		+				+		
19. Prionospio cirrifera	+	+			+	+	+							+	+	
20 Platynereis dumerelii																+
21. Pectinaria sp.																+
22. Polydora ciliata				+												
23. Syllis hyaline	+															
24. Terebellides stroemi	+											+	+			
Olygochaeta																
1. Olygochaeta sp.										+						
Tentaculata - Phoronidae																
1. Phoronis euxinicola		+					+									
Arthropoda Crustacea																
1. Ampelisca diadema		+				+	+						+		+	
2. Apseudopsis ostroumovi																+
3. Balanus improvisus	+	+	+	+	+		+	+	+	+	+	+	+	+	+	
4. Brachinotus sexdentatus	+			+												+
5. Callianassa pestai	+															
6. Cymacea sp.										+						
7- Clibanarius erythropus										+	+					
8. Crangon crangon																+
9. Corophium crassicome														+		
10. Diogenes pugilator	+	+	+			+	+	+		+	+		+	+		+
11. Gammarus (Marinogammarus olivii)	+	+					+						+			
12. Gammarus subtyPicus																+
13. Hyale pontica				+												
14. Indotea baltica basteri																+
15. Macroppuls depurator	+									+						+
16. Pisidae longimana																+
17. Potamon potamios																+
18. Palaemon elengans																+
19. Siriella jaltensis ialtensis		+			+	+	+								+	

20. Upogebia pusilla								+								
21. Xontho poressa																+
Mollusca GastroDoda																
1. Cyclope donovani	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
2. Cylichnina robagliana			+													
3. Cylichnina strigella		+		+				+	+	+			+	+	+	
4. Cylichnina variabilis				+				+			+					
5. Caluptraca chinensis																+
6. Caecum elegans															+	
7. Chrysallida incerta	+			+												
8. Hydrobia leneumiere															+	
9. Hydrobia acuta								+								
10. Nana neritea																+
11. Odostomia albella																+
12. Odostomia pallida		+		+				+		+			+	+	+	
13. Odostomia acuta														+		
14. Parthenina terebelides													+			
15. Parthenina interstincta		+		+					+		+		+	+	+	
16. Rapana thomasiana	+	+								+						+
17. Retusa truncatella								+								
18. Tritia reticulata	+												+			+
19. tricolia pulla	+	+			+						+		+	+		
Lamellibranchiata s. Bivalvia																
1. Abra nitida milachewichi	+			+	+			+		+	+	+			+	+
2. Arca noae	+	+	+	+			+	+		+	+		+	+		+
3. Cunearca cornea	+	+		+				+	+	+	+	+		+	+	+
4. Cerestoderma glaucum				+											+	
5. Chamelea gallina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6. Gouldia minima	+				+				+						+	
7. Donax trunculus											+	+	+			
8. Donacila cornea								+								
9. Hypanis sp.								+								
10. Lentidium mediterraneum	+	+		+		+	+	+	+	+			+	+	+	
11. Lucinella divaricata																+
12. Mutilus galloprovincialis	+	+		+			+			+	+		+	+		+
13. Modiolus phaseolinus		+												+		+
14. Modiolus adriaticus																+
15. Mocrella donacina																+
16. Mytilaster lineatus										+						
17. Polititapes aurea		+		+	+									+	+	
18. Pitar rudis	+			+	+					+	+			+		
19. Spisula subtruncata		+	+		+		+	+					+	+	+	
20. Spisula triangular	+	+		+	+	+	+	+	+	+	+		+	+		
Echinodermata																
1. Amphiura stepanovi	+	+	+	+	+	+	+	+	+	+	+	++	++	+	+	+

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შავი ზღვის შელფის (გონიო-ქობულეთი) ბენთოშაუნის მონიტორინგი

გოგმაჩაძე თ., მიქაშავიძე., დიასამიძე რ.

ზოოლოგიის კათედრა, შ. რუსთაველის სახ. ბათუმის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

მონიტორინგის შედეგად დადგენილია, რომ საკვლევი რეგიონის შელფზე დომინირებს ხამელეას (*Chamelea gallina*) ბიოცენოზი სილის, შლამიანი სილის და შლამის სუდიმენტებით. ბენთოსი წარმოდგენილია 92 სახეობის უხერხემლო ცხოველებით, რომლებიც მიეკუთვნებიან რამოდენიმე მსხვილ ტაქსონს. ცენოზწარმომქმნელი სახეობა *Chamelea gallina* არის ლაყუჭფირფიტიანი ანუ ორსადგულიანი მოლუსკი. გარდა ამ სახეობისა დომინანტებს და სუბდომინანტებს მიეკუთვნება სამი ორსადგულიანი მოლუსკი (*C.cornea, S.triangular, L.mediterraneum*) და თითო სახეობა მუცელფეხიანი მოლუსკებიდან (C.donovani), კიბოსნაირებიდან (*B.improvisus*) და კანქლიანებიდან (*A.stepanovi*). აქედან *Cunearca cornea* ამ რეგიონში პირველად ჩვენს მიერ იქნა რეგისტრირებული

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SPECIES COMPOSITION AND DYNAMICS OF EARTHWORMS (*OLIGOCHAETA, LUMBRICIDAE*) IN CONIFEROUS FORESTS OF THE ALGETHY RESERVE

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(Received April 19, 2004)

Abstract

Species composition and quantitative dynamics of five association of earthworms in spruce forests and six association in pine forests were studied. 10 species of earthworms were registered in spruce forests and 13 – in pine forests. The density of earthworms in pine forests is much higher than in spruce forests, while pine forests grow on well developed black forest soils and spruce forests – on stony soils. The dynamics of earthworms is similar both in spruce and pine forests.

Key words: Earthworm, Algethy Reserve, East Georgia, Quantitative dynamics.

Introduction

The Algethy Reserve is located on the lower slopes of eastern part of Trialeti Range, in the basin of upper part of the Algethy River. The Algethy Reserve spreads over 6 822 hectares. The main part (88,5%) of the reserve is covered by forests. The main components of the coniferous forests are spruce – covering 1 442 ha, pine – 250 ha and fir – 1 ha. It is noticeable, that the Algethy Reserve is an eastern border of the area of oriental spruce (*Picea orientalis*) and the Caucasus fir (*Abies nordmaniana*) [Chincharauli, 1988].

The biodiversity of the Algety Reserve invertebrates and earthworms among them was not studied. The role of the earthworms in the soil fertilization and functioning of ecosystems is very important. So, the species composition and density of earthworms of the Algety Reserve coniferous forests have been researched.

Material and methods

Investigation of the earthworms were carried from May 2001, till June 2003. Accepted methods in soil zoology were used (stationary and rout methods) [Giliarov, 1964]. Stationary method was used in the spruce forest with moss cover (*Piceetum muscosum*) on the right bank of the Algethy River and in the pine forest (*Pinetum carpinolosum*) near Manglisi.

Rout methods [Chincharauli, 1988] were used in different associations of spruce and pine forests from elevation 1 200m. Namely:

1. Spruce forest (*Piceetum muscosum*) on the right bank of the Algethy River;
2. Spruce forest (*Piceetum caricosum*) in Rusi's Forest;

3. Spruce forest (*Piceetum nudum*) – Namtvriani;
4. Spruce forest (*Piceetum poosum*) – Kldekari;
5. Spruce forest (*Piceetum festucosum*) – Chinchriani.

Stationary methods were used in following forests:

1. Pine forest (*Pinetum carpinolosum*);
2. Pine forest (*Pinetum prachypoolioso-caricosum*) – Tkhinvala;
3. Pine forest (*Pinetum brachypodiosum*) – Mzhavisi;
4. Pine forest (Pinetum brachypodioso-caricosum) – Ukhmara;
5. Pine forest (*Pinetum brachypodioso-calamagrasticisum*) – Chinchriani;
6. Pine forest (*Pinetum hylocomiosum*) – Ugudeti.

Results and Discussions

Fir forests create small "islands" in the Algethy Reserve and fir trees in this groups are presented together with beech, hornbeam and other species of trees. Earthworms were studied mainly in spruce and pine forests, because fir forests cover insignificant area of the Algethy Reserve.

In the spruce forests 10 species of earthworms were registered [Tsiklauri, 2004]. Maximal density – 24 n/m^2 was observed on the right bank of the Algethy River and minimal – 1,3 n/m^2 in Namtvriani. Comparably high density of earthworms (10,6 n/m^2) was found in Russi's forest.

Dominant species are: Dendrobaena surbiensis, D. tellermanica, Allobophora caliginosus trapezoides.

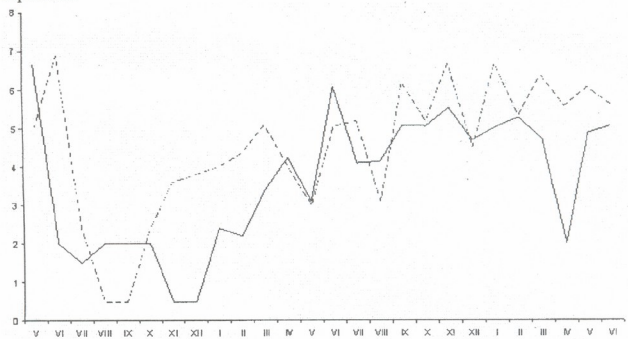

Fig. 1. —— dynamics of the spruce forests earthworms, - - - - dynamics of the pine forests earthworms.

In the pine forest soils 13 species of earthworms were found. The highest density (38,7 n/m^2) was registered in Tkhinvala, followed by the pine forest near Manglisi (37,2 n/m^2). The minimal density of earthworms (9,3 n/m^2) was observed in Mzhavisi.

Pine and spruce forests of the Algethy Reserve have similar dominant species of earthworms and the quantitative dynamics has similar alternative variations with insignificant changes, that is observed in mezofauna (Fig. 1).

From May 2001 till May 2002 the number of earthworms of pine and spruce forests changed equally. During this period maximal density was observed in spring and next maximum in summer 2002 and 2003 in both formations.

In summer 2002 the dynamics of earthworms are characterized by numerous alternative variations in the pine forests, different to those in the spruce forests.

The minimal number (1,3 n/m^2) in the pine forests was observed in August and September 2001. In the spruce forests decrease of density was observed in autumn (October, November), but in December density didn't chang. According to our investigation, number of earthworms decreases during seasonal changes.

Thus, the number and variety of species of earthworms are higher in the pine forest soils than in the spruce forest soils. The reason is that in the Algethy Reserve spruce oriental (*Picea orientalis*) "is attracted not to the favorable biotopes, but to the stony and rocky places with dry, weakly developed soils" [Dolukhaniv, 1977].

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ალგეთის ნაკრძალის წიწვოვანი ტყეების ჭიაყელების სახეობრივი შემადგენლობა და დინამიკა

წიკლაური ხ., ყვავაძე ე.

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(მიღებულია 19.04.2004)

რეზიუმე

სტაციონარული და მარშრუტული მეთოდებით შესწავლილია ნაძვნარების ხუთი და ფიჭვნარების ექვსი ასოციაციის ჭიაყელების სახეობრივი შემადგენლობა. ნაძვნარებში აღირიცხა ჭიაყელების 10 სახეობა, ხოლო ფიჭვნარებში – 13. ჭიაყელების დასახლების სიმჭიდროვე გაცილებით მაღალია ფიჭვნარებში, ვიდრე ნაძვნარებში, რაც იმით აიხსნება, რომ ალგეთის ნაკრძალში ნაძვი იზრდება ხირხატიან ნიადაგებზე, ხოლო ფიჭვი – ტყის კარგად განვითარებულ ყომრალ ნიადაგებზე. ნაძვნარებსა და ფიჭვნარებში ჭიაყელების რიცხოვნობის დინამიკის საერთო სურათი მსგავსია.

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Genetics

THE STUDY ON POLYMORPHISM OF C HETEROCHROMATIN IN PATIENTS WITH UNDIFFERENTIATED FORM OF OLYGOPHRENIA

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(Received May 10, 2004)

Abstract

The polymorphism of heterochromatin of the undifferentiated form of Olygophrenia according to the variants of C-structural heterochromatin blocks has been studied. The heterogenicity was defined for 1, 9 and 16 chromosomes. 1 and 9 chromosomes were characterized by decline in large variants of C-blocks, while 16 chromosome was characterized with sharp increase in the largest variant of C-heterochromatic blocks.

Key words: chromosome, heterochromatin, polimorphism, C-bands, Olygophrenia.

Introduction

It is known, that there is a link between the alterations of human's morphophysiological properties and genome instability in case of certain pathologies. it has been stated that the variability of normal features and the development of pathologies are connected with chromosomal polymorphism. The special variants of chromosomal polymorphism correlate with phenotypic characteristics and pathological state. In case of pathologies the most important is to reveal the caryotypes, that contain extremal variants of each chromosome, especially it deals with 1, 9 and 16 chromosomes [Kovalova et al.,1983; Kuznietsova et al., 1996]. The biggest heterochromatic blocks are localized in these chromosomes. Their sizes subordinate to the quantitative evaluation and they vary within the vast ranges during normal and pathological state.

Olygophrenia is defined as a pathology in the development of which the role of genotype has been clearly determined. The differentiated forms of the mentioned pathology group are Down and Martin Bell's syndromes. The mentioned syndromes are characterized by the variability of structural heterochromatin [Podugolnikova et al., 1987; Kuznetsova et al., 1996]. The main goal of our research was to study the polymorphism of the chromosomes C-heterochromatin regions in the individuals with undifferentiated form of Mental Retardation.

Material and Methods

Investigations have been carried out on peripheral blood lymphocyte cultures derived from Mental Retarded (M.R.) children at the age 7-15. The structural C – heterochromatin polymorphic variants frequently were detected in chromosomes 1, 9 and 16. Sumner's modified method has

been used to reveal these blocks. The C – segments variants were determined by classification of Patil and Lambs.

a<0,5x16p; b>0,5 – 1x16p; c>1,5x16p; d>1,5 – 2x16p; e>2x16p;

Statistic analyses has been carried out by Zax formula:

$$x^{2}_{(k-1)} = (n+m)\frac{n}{m}\left\{\sum_{i=1}^{k} = \frac{\left(\frac{V_i}{n}\right)^{2}}{\frac{V_i+\mu_i}{n+m}} - 1\right\}$$

V_i - number of a,b... or e variants in control group;

μ_i - number of a,b... or e variants in cells of children with M.R.;

n – total number of C-bands variants in control group;

m – total number of C-bands variants in cells of children with M.R.;

Results and Discussion

On the first stage the quantitative analysis of 1, 9 and 16 chromosomes C-segments has been carried out in the individuals with Mental Retardation and healthy controls [Bablishvili et al., 2000] (Table 1.). As the result of our investigations, the increased frequency of B-variants of C-segments has been revealed in patients comparatively to control group. The meaning of x^2 has been decreased x^2_3=29,83; p<0,01. As for different variants of C-heterochromatic blocks their frequencies as for the frequencies of various C-heterochromatic determined in our control group [Akopiani, Buzhievskaia 1986; Maligina et al., 1988; Bablishvili et al., 2000]. They are in agreement with scientific data.

Heteromorphic analysis of C-segments for each 1, 9 and 16 chromosomes has been carried out as well (Table 2.). It has been discovered that mean size of c-variant of heterochromatic blocks frequently was localized on the first chromosome in patients with Mental Retardation (the frequency of b-variants was the highest) (Table 1.). C-variant as well as b is characterized by high frequency in the control group. The localization frequency of a- and b-variants on the first chromosome was significantly lowered with controls x^2_3=10,14; p<0,01. As for the frequency of d-variant segments it was significantly increased in the individuals with Mental Retardation.

Table 1. Polimorphism of C-segments in patients with undifferentiated form of Olygophrenia

Variants of C-segments	V_i	μ_i	$\frac{V_i}{n}$	$\frac{\mu_i}{m}$	$\frac{V_i+\mu_i}{n+m}$	x^2
a	144	150	0,2553	0,2836	0,2836	
b	267	199	0,4734	0,3763	0,4263	$x^2_3 = 29,83$
c	136	125	0,2411	0,2363	0,2388	$p < 0,01$
d	17	53	0,0301	0,1002	0,0640	
e	-	-	-	-	-	

Table 2. Polymorphism of C-bands on chromosomes 1, 9, 16 with undifferentiated form of Olygophrenia

Chromosomes	Variants of C-segments	V_i	μ_i	$\frac{V_i}{n}$	$\frac{\mu_i}{m}$	$\frac{V_i + \mu_i}{n+m}$	x^2
1	a	17	1	0,0876	0,0057	0,0488	$x_3^2 = 10,14$ $p < 0,01$
	b	79	37	0,4072	0,2114	0,3171	
	c	79	86	0,4072	0,4914	0,4472	
	d	19	51	0,0979	0,2914	0,1897	
	e	-	-	-	-	-	
9	a	39	16	0,2042	0,0919	0,1507	$x_3^2 = 3,87$ p>0,05
	b	99	120	0,5183	0,6897	0,6	
	c	50	38	0,2618	0,2184	0,2411	
	d	3	-	0,0157	-	0,0082	
	e	-	-	-	-	-	
16	a	88	133	0,4783	0,7430	0,6088	$x_3^2 = 15,54$ p<0,01
	b	89	42	0,4837	0,2346	0,3609	
	c	7	1	0,0380	0,0056	0,0220	
	d	-	2	-	0,0112	0,0055	
	e	-	-	-	-	-	

The frequency of b-variant localization was the highest on the 9th chromosome as compared with 1st chromosome, the same variant appeared in control as well but the frequency index was relatively decreased than in case of Olygophrenia. The frequencies of c- and a- variants were significantly decreased for 9th chromosome in comparison with control group. According to the variants of C-heterochromatic blocks the heterogenicity has revealed for 16th chromosome in MR patients. The highest frequency of C-blocks a-variant was identified on 16th chromosome, the index was significantly increased as compared with healthy individuals, x_3^2 =15,54; The frequency of all variants were decreased in comparison with control.

The result of our research was chromosomal polymorphism revealed in individuals with undifferentiated form of Olygophrenia according to the variants of C-structural heterochromatin blocks. The heterogenicity was defined for 1, 9 and 16 chromosomes. According to the recent scientific data the polymorphism of heterochromatin is also the characteristic of the differentiated form of Olygophrenia. Thus, in this case the high frequency of small variants is noticed in karyotype compared with control. Our results suggest that in some cases the frequency of large a-variant of undifferentiated of 1, 9 and 16 chromosomes in comparison with control group, significantly increases (for 16 chromosome). We suppose that the study on polymorphism of C-structural heterochromatin might be perspective for the classification of undifferentiated forms of Olygophrenia.

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C-სტრუქტურული ჰეტეროქრომატინის პოლიმორფიზმის შესწავლა ოლიგოფრენიის არადიფერენცირებული ფორმების შესწავლის დროს

დადუნაშვილი ე., ჯოხაძე თ., ბაბლიშვილი ნ., თადუმაძე ნ., დიდებულიძე დ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

შესწავლილ იქნა C-სტრუქტურული ჰეტეროქრომატინის პოლიმორფიზმი ოლიგოფრენიის არადიფერენცირებულ ფორმაში ჰეტეროქრომატინის ბლოკების ვარიანტების მიხედვით. ჰეტეროგენურობა დაფიქსირდა სამივე წყვილი 1-ლი, მე-9 და მე-16 ქრომოსომებისთვის. 1-ლი და მე-9 ქრომოსომებისთვის ძირითადად დამახასიათებელი იყო ბლოკების დიდი ზომის ვარანტების დაქვეითება, მე-16 ქრომოსომაზე კი აღინიშნება C-ჰეტეროქრომატინის ბლოკების მაქსიმალური ვარიანტის სიხშირის მკვეთრი ზრდა.

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CHROMOSOMAL NUCLEOLAR ORGANIZING REGIONS (NORS) IN PATIENTS WITH DIFFERENT FORMS OF THYROID DISEASES

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(Received April 14, 2004)

Abstract

With the aim of assessing transcriptional activity in lymphocytes, the blood samples from 34 patients with different forms of thyroid lesions (diffuse-toxic, endemic, sporadic, nodular-toxic, autoimmune thyroiditis and congenital hypoplasia) were cultured and stained with silver nitrate for nucleolar organizing regions (NORs). Mean frequency rate of argentopfilic satellite associations, number of silver-positive acrocentric chromosomes per cell and NOR size-wise distribution varied among different forms of thyroid disorders indicating the state of activity of the ribosomal genes located there. Significant deviations in activity parameters from control values were recorded for all studied forms. Decreased proportions of cells that contained acrocentric chromatid associations were found in all studied forms of thyroid disorders with an exception of nodular-toxic form. The lowest number of Ag-positive non-associated acrocentric chromosomes per cell was recorded in cases of autoimmune thyroidit and endemic forms of thyroid diseases (3.81 and 3.98, correspondingly), whereas diffuse-toxic and nodular-toxic forms had a higher number of AgNOR counts (5.05 and 5.03 respectively) compared to the control value (4.18).

Key words: thyroid lesion; transcriptional activity; NORs; Ag-staining; chromatid association.

Introduction

The nucleolar organizing regions (NORs) contain highly repetitive ribosomal RNA genes as well as rRNA transcripts and associated proteins. They are located at the secondary constrictions of ten acrocentric chromosomes of D and G groups and are stained by silver-staining technique. The number of Ag-stained chromosomes as well as the amount of silver stain on each acrocentric chromosome and the rate of satellite associations vary from person to person in dependence with the transcriptional activity of ribosomal cistrons [Trere, 2000]. These parameters reflect the intensity of synthetic processes in individuals and are successfully applied in cytogenetic

investigations performed at different stages of ontogenesis [Lezhava, 2001]; in studies on the effect of various endogenous and exogenous factors, medicines and bioregulators [Khavinson et al., 2002; Lezhava et al., 2004] on cell functioning; in patients suffering from different diseases [Narayan et al., 1998; de Silva et al., 2000; Zosidze, Koplatadze, 2003].

In the present study an attempt has been made to assess some forms of thyroid diseases (diffuse-toxic, endemic, sporadic, nodular-toxic, autoimmune thyroidit and congenital hypoplasia) from the view point of transcriptional activity of NORs in lymphocytes obtained from the patients.

Materials and Methods

For the present study in total 34 patients with 6 forms of thyroid diseases (diffuse-toxic, endemic, sporadic, nodular-toxic, autoimmune thyroidit and congenital hypoplasia) were examined. Residents of mountainous regions of Adjara made up the endemic group. Three of the patients were re-examined after passing 3-month-course of treatment. For each case, 30-50 intact metaphases were examined for NOR activity. 7 middle-aged healthy individuals (360 cells) were used as controls. Metaphase chromosome preparations were made from 72-hrs lymphocyte cultures (ISCN, 1985) and were silver-stained by a slight modification of the standard method [Bloom, Goodpasture, 1976]. Number of Ag-positive NORs and the frequency of entering satellite associations by acrocentric chromosomes of D and G groups were tested. It should be noticed that the chromosomes without silver bands do not enter the associations. NOR activity was estimated by the size of Ag-bands using scale units of 0-2.

Results and Discussion

The data obtained from the analysis of argentophilic satellite associations are given in Fig.1. Decreased proportions of cells that contained acrocentric chromatid associations were found in all studied forms of thyroid disorders (61.33% for diffuse-toxic; 39.48% - endemic; 37.58% - sporadic; 45.2% - autoimmune thyreoidit; 23.0% - congenital hypoplasia) with an exception of nodular-toxic form (71.5%) as compared to the mean control value (73.6%). The evaluation of silver-stained NOR sizes revealed a positive correlation – the frequency of associations increased in parallel to staining scores. the rate of satellite associations (mostly containing only two acrocentrics) varied from person to person within each group of patients, however the range of variations were not wide.

Numbers of total and chromosome group-specific types of associations (DD, DG and GG) per cell are shown in Fig 2. The incidence of acrocentric chromosome associations per cell was lowest in the case of congenital hypoplasia (0.24) and highest at nodular-toxic form (1.05; control value – 0.79 per cell).

We have also evaluated the incidence of so called 'opened' associations. The latter represents chromosome attachment by Ag-positive intersatellite stalks with free satellites and it may be presumed to be a functional analogue of a single acrocentric chromosome with Ag-positive NOR [Lezhava, 1999]. In all studied forms the portion of 'opened' associations was miserable (within the range of 0.05 – 0.4 per cell), at a quite high rate in control subjects – 0.79.

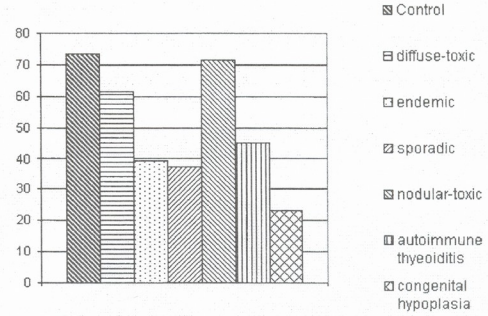

Fig. 1. Percentage of cells with acrocentric chromatid associations at different forms of thyroid disorders. On abscess axis – forms of thyroid lesion

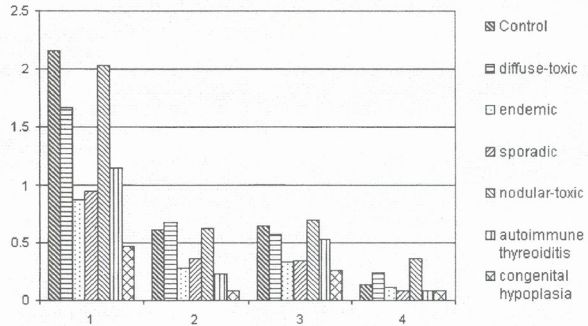

Fig. 2. Frequency of total and chromosome group-specific types of associations per cell on abscess axis – total number of associations (1), types of associations: DD (2), DG (3), GG (4).

If satellite associations occur randomly, the ratio of D-group to G-group chromosomes in these associations should be 6:4, unless there are presented abnormal numbers of D- and G-group chromosomes . In our controls, the corrected ratio of G-group to D-group chromosomes in association per cell (D/G) was 1.84. For different forms of thyroid lesion the ratios were as follows: 1.69 for diffuse-toxic; 1.56 - endemic; 1.94 - sporadic; 1.39 - nodular-toxic; 1.45 - autoimmune thyroidit; 0.96 - congenital hypoplasia. In the latter case number of G-group chromosomes in satellite association was greater than that of a D-group chromosome.

The lowest number of Ag-positive non-associated acrocentric chromosomes per cell was recorded in cases of autoimmune thyrodit and endemic forms of thyroid diseases (3.81 and 3.98,

correspondingly), whereas diffuse-toxic and nodular-toxic forms had a higher number of AgNOR counts (5.05 and 5.03 respectively) compared to the control value (4.18). The frequency of chromosomes with NOR sizes scoring 2 in all the studied forms was higher (varied from 2.09 to 3.67 per cell) as compared to the control parameter (1.75). We suppose that enlarged AgNOR counts in cells obtained from thyroid patients should be considered as an additional compensatory mechanism for transcription against a background of declined associative activities of acrocentric chromosomes.

Three of the patients (with congenital hypoplasia, diffuse-toxic and nodular-toxic forms) were re-examined for NOR activity after they had undergone 3-month-course of treatment. Some of the studied parameters were found to become more or less corrected by an effective therapy, so that they returned to levels similar to those observed in healthy individuals.

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Genetics

VARIABILITY OF UNSCHEDULED DNA SYNTHESIS INDUCED BY NICKEL IONS AND PEPTIDE BIOREGULATOR EPITALON IN OLD PEOPLE

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Abstract

The effects of Nickel Chloride and peptide bioregulator Epitalon on the unscheduled DNA synthesis intensity in cell cultures derived from individuals at the age of 76-81 have been studied. Our results indicate that: 1. The repression of unscheduled DNA synthesis takes place in aged individuals (repair index – 0,85); 2.The low level of unscheduled DNA synthesis characterising old individuals is far more decreased in case of treatment with Nickel Chloride (repair index – 0,62); 3. Epitalon sharply increases the level of unscheduled DNA synthesis in old individuals (repair index – 2.15).

Key words: aging, chromatin condensation, Epitalon, heterochromatinization, repair, unscheduled DNA synthesis.

Introduction

According to the experimental data, the modification of DNA-repair processes is possible towards both directions: to weaken or enhance their intensity. The first event is mainly reached using inhibitors that suppress the activity of certain repair enzymes i.e. substances of this group basically inhibit separate repair processes provoking increased level of mutagenesis. This process also has an influence on the effectiveness of repair intensity when various anticlastogenic compounds are used.

It is also known that the condensation of nucleosomes in highly organised eukaryotes which progresses in relation with age, is able to limit the activity of excision repair enzymes within the damaged sites [Lezhava, 2001a]. Taking into account this suggestion, the chromatin of old individuals represents an available model system because the main reason for decreased repair intensity and the development of aging pathologies, is progressive heterochromatinization (chromatin condensation) taking place during the process of aging [Lezhava, 2001b]. Therefore to find out different ways of the effectiveness of repair system, the usage of such compounds that have an ability of chromatin modification would be perspective [Jokhadze and Lezhava,1994; Goldstein et al., 2003].

The goal of our research was on the one hand, to study the influence of Nickel Chloride on DNA – repair system (unscheduled DNA synthesis) intensity in cell cultures derived from old individuals (nickel chloride also increases the level of chromatin condensation) and on the other hand, to investigate the activity of such compound that has an opposite effect-chromatin decondensation. For this reason we have selected a synthetic peptide bioregulators [Khavinson et

al., 2004, Lezhava et al., 2004], among them Epitalon, that provides the activation of chromatin [Khavinson et al., 2002, 2003].

Material and Methods

Object of research. The studies were performed on PHA-stimulated and non-stimulated peripheral blood lymphocytes derived from healthy donor's aged 76 to 81 (10 individuals) and donor's 20 to 43 yrs. (6 individuals). The solutions of nickel chloride and peptide Epitalon were introduced in the stimulated cultures after 24 hours from their cultivation. In case of non-stimulated cultures the solutions were added immediately and cultures were retarded for 18-20 hours.

Epitalon. Peptide bioregulator Epitalon was obtained by targeted chemical synthesis from Epithalamin in the St.Petersburg Institute of Bioregulation and Gerontology of the Russian Academy of Medical Sciences. It stimulates immune processes, prevents premature aging, decreases the probability of aging pathologies and it is successfully adopted in medical practice.

Mutation. Chromosomes aberrations were studied in 500 metaphases from 5 individuals of age from 19 to 25. 10^{-4} M of nickel chloride was added in 72h to the lymphocytes cultures. Cultivation of human lymphocytes, cells aberration analysis were carried out according to the International System for Human Cytogenetic Nomenclature (ISCN) and performed as described by Lezhava [Lezhava, 1999)

Repair . Unscheduled DNA synthesis has been investigated in UV-irradiated lymphocytes by the method of Lezhava [Lezhava, 1999]. Heparinized venous blood from clinically normal individuals of both sexes was used; the study group involved 10 individuals aged from 76 to 81, and the control group included 6 individuals aged 20 to 43. 1.5 ml of lymphocyte-containing plasma was put into incubation flasks (final lymphocyte concentration was 1×10^6 per ml). Each flask contained 3 ml of the culture medium. Oxyurea (10 mole per ml of the culture), as an inhibitor of DNA replicative synthesis was used. Therefore, DNA synthesis registered by us was defined as unscheduled repair synthesis. The cultures were incubated for 30 min at 37 °C, and 1.5-ml aliquots were poured into Petri dishes. The lymphocyte-containing fluid layers were 1 mm thick. The cultures were exposed to UV irradiation, 15 J/mm^2, using a bactericidal lamp (λ = 254 nm; capacity-1.98 J/mm^2/s). Immediately after irradiation H^3-thymidine was introduced into the medium (final concentration - 10 μCi/ml; specific radioactivity - 14 Ci/mmole). The cultures were incubated in centrifuge tubes for 2.5 hours at 37 °C and then centrifuged at 800 g for 5 min at room temperature. Supernatants were removed, and 0.5 ml of Haenks' solution was added to sediment. Cells were carefully resuspended and transferred onto filters. The filters were dried, washed three times with 5 percent trichloracetic acid cooled to 4 °C, rinsed with absolute alcohol and dried. Radioactivity was measured in 5 ml of the
toluol scintillation fluid using a scintillation counter.

Results and Discussion

Chromosomal Aberration. On the first stage of research we have studied the influence of Nickel Chloride on the frequency of structural chromosomal disorders. In case of Nickel chloride, its 10^{-4} M solution has been tested. i. e. the concentration that did not induce mutations. Noticeabely, the cells of old individuals are characterised by high spontaneous level of chromosome aberrations and it was revealed in our studies as well. As for nickel, it sharply increased the frequency of chromosome aberrations [Cangul et al., 2002]. An average frequency of

aberration – containing cells was equal to 25,5% (in intact cultures – 5,1% ±1,2%). Scientific data vary in accordance with model systems [Lezhava et al., 2001b]. In case of Epitalon its concentration corresponded to the one-fold therapeutic dose – 0,005 mg/ml, that is not characterised by mutagenic activity.

Unscheduled DNA synthesis. On the next stage we have studied the influence of nickel chloride and Epitalon on the intensity of DNA-repair processes. It is known that one of the indices of excision repair level is unscheduled DNA synthesis or relatively weak synthesis. It appears as a response of influence of either UV-irradiation or such chemical agents that cause DNA damages in non S-phase cells.

Using UV dosages of 15 J/mm^2, the rates of unscheduled DNA synthesis at aging were considerably lower - 389 impulses per minute and for the control group (20 to 43 years) - 456 impulses per minute.

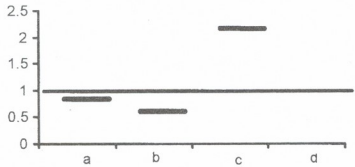

Fig. 1. unscheduled DNA synthesis by UV-irradiation dosages of 15 J/ mm^2 in aging lymphocytes with effects of nickel chloride and peptide Epitalon.
a – repair index of old individuals + UV; b – repair index of old individuals + UV + nickel chloride; c – repair index of old individuals + UV+ Epitalon; a-d – repair index value - 1.00, indicated that there was no detectable repair synthesis.

To study the modification ability of nickel ions and Epitalon we used repair index as a depictor of unscheduled DNA synthesis level, i.e. repair index (the ratio of isotope label incorporation in experiment and control) was not detected. In aged lymphocytes the repair index induced by UV-irradiation was equal to 0,85 ($p< 0,001$). Nickel chloride and Epitalon significantly changed the level of recovery induced by UV-irradiation. Their influence was not identical. Nickel chlorid significantly lowered the repair index in aged cells – 0,62 (283 – impulses/minute) ($p< 0,001$), while without Epitalon had an opposite effect - 2,15 ($p< 0,001$) (980 – impulses/ minute).

As mentioned above nickel ions increase the degree of chromatin condensation and therefore it is no more accessible for repair enzymes, that is the reason for the decreased level of UV- induced repair synthesis. In case of Epitalon the maximal repair index was revealed – 2,15. Epitalon is only the modifcator of UV-induced repair synthesis. According to the data Epitalon engenders chromatin decondensation in cells derived from old individuals [Khavinson et al., 2003]. This factor plays the main role in increased activity of repair enzymes and correspondingly the repair index increases.

On the bases of our results we can conclude: 1.The repression of unscheduled DNA synthesis takes place in aged individuals (repair index 0,85); 2. The low level of unscheduled DNA synthesis characterising old individuals is far more decreased in case of treatment with nickel chloride (repair index –0,62). 3. Epitalon sharply increases the level of unscheduled DNA synthesis in old individuals (repair index 2,15).

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დმნ-ის არაგენმური სინთეზის ცვალებადობა ნიკელის იონებით და ბიორეგულატორ ეპიტალონით ზემოქმედებისას ხანდაზმულ ინდივიდებში

ლეჟავა თ., ჯოხაძე თ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

შესწავლილია ნიკელის ქლორიდისა და პეპტიდური ბიორეგულატორის ეპიტალონის გავლენა დნმ-ის არაგენმური სინთეზის ინტენსივობაზე 76-81 წლის ინდივიდთა უჯრედულ კულტურებში. მიღებული შედეგები მიუთითებენ: 1.ხანდაზმულ ინდივიდებში ადგილი აქვს დნმ-ის არაგენმური სინთეზის დათრგუნვას (რეპარაციის ინდექსი – 0,85); 2. ხანდაზმული ინდივიდებისათვის დამახასიათებელი დნმ-ის არაგენმური სინთეზის დაბალი დონე ნიკელის ქლორიდით ზემოქმედებისას მნიშვნელოვნად ქვეითდება (რეპარაციის ინდექსი – 0,62); 3. პეპტიდური ბიორეგულატორი ეპიტალონი მკვეთრად ზრდის დნმ-ის არაგენმური სინთეზის დონეს ხანდაზმულ ინდივიდებში ნიკელის ქლორიდით ზემოქმედებისას (რეპარაციის ინდექსი – 2,15).

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Genetics

INFLUENCE OF EPITALON ON GENETIC ACTIVITY OF HEAVY METAL SALTS ($PB(NO_3)_2$, $CDCL_2$, $NICL_2$) IN *DROSOPHILA MELANOGASTER*

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(Received October 20, 2003)

Abstract

The combinative treatment of 1 mkg Epitalon and high mutagenic doses (10^{-1}M) of heavy metal salts ($Pb(NO_3)_2$, $CdCl_2$ and $NiCl_2$) was studied in *Drosophila melanogaster* by the method "Meller – 5" assessing sex-linked recessive lethal and sublethal mutations. Epitalon in combination with heavy metal salts revealed the strongly decreased mutagenic activity in comparison with the action of single heavy metal salts. Epitlon also decreased the number of wing – morphoses induced by heavy metal salts in *Drosophila melanogaster*. It was supposed that Epitalon has the antimutagenic properties.

Key words: drosophila, heavy metals, epitalon, mutations

Introduction

In recent years new preprations were introduced in gerontologic and geriatrics practice as medical bioregulators – Epitalon, Vilon and Prostamax. These compounds were found to have geroprotective effects. They perform peptide regulation of organism, improve the functional indices of endocrine, immune and nervous systems, homeostasis and metabolism. They are used to strengthen prevention ability of organism against unfavourable ecological, climatic and other environmental factors [Khavinson V. K., et al., 2002].

Material and methods

The goal of our work was to study the Influence of Epitalon on genetic activity of heavy metal salts in *Drosophila melanogaster*.

The standard method of "Meller-5" was used to register the sex-linked recessive lethal and sublethal mutations in *Drosophila melanogaster*.

The high mutagenic doses of heavy metal salts - $Pb(NO_3)_2$, $CdCl_2$, $NiCl_2$ (10^{-1}M for each compounds [Nibladze N., et al., 2003] were used in our experiment. The combinative action of 1

mkg dose of Epitalon and 10^{-1}M dose of each heavy metal salts was studied. The exposition time lasted 24 h. The nutrient medium was served as a solvent.

Results and discussion

As it is shown in Table 1 (Fig. 1), the combinative action of Epitalon and heavy metal salts decreased two-fold and more the mutagenic activities of single heavy metal salts in *Drosophila melanogaster* – after treatment with single - $Pb(NO_3)_2$, $CdCl_2$ and $NiCl_2$ the total number of sex-linked recessive lethal and sublethal mutations equaled to 16,03%, 13,66% and 11,53% - respectively, while in case of combinative action of Epitalon and heavy metal salts – 9,32%, 5,63% and 6,12% - respectively.

Table 1. The frequency of sex-linked recessive lethal and sublethal mutations after treatment with single heavy metal salts and in combination with Epitalon

compound and concentration	invest. crom. numb.	mutation frequency					
		lethal		sublethal		Total	
		n	% ± m	n	% ± m	n	% ± m
Epitalon+10^{-1}M $Pb(NO_3)_2$	236	8	3,39± 1,18	14	5,93± 1,54	22	9,32± 1,89
10^{-1}M $Pb(NO_3)_2$	212	14	6,60± 1,71	20	9,43± 2,00	34	16,03± 2,52
Epitalon+10^{-1}M $CdCl_2$	231	6	2,59± 1,05	7	3,03± 1,13	13	5,63± 1,52
10^{-1}M $CdCl_2$	227	12	5,29± 1,48	19	8,37± 1,84	31	13,66± 2,28
Epitalon+10^{-1}M $NiCl_2$	245	7	2,86± 1,06	8	3,27± 1,14	15	6,12 ± 1,53
10^{-1} M $NiCl_2$	234	10	4,27± 1,32	17	7,26± 1,70	27	11,53± 2,09
Epitalon	236	0	0	0	0	0	0
control	255	0	0	0	0	0	0

Table 2. The frequency of wing morphoses after treatment with single heavy metal salts and in combination with Epitalon

compound and concentration	invest. crom. numb.	morphosis frequency							
		long-winged		single-wing-open		double-wing-open		Total	
		n	% ± m	n	% ± m	n	% ± m	n	% ± m
Epitalon+10^{-1}M $Pb(NO_3)_2$	4720	0	0	28	0,59± 0,11	20	0,42± 0,09	48	1,02± 0,15
10^{-1}M $Pb(NO_3)_2$	4240	71	1,67± 0,19	16	0,38± 0,09	13	0,31± 0,09	100	2,36± 0,23
Epitalon+10^{-1}M $CdCl_2$	4620	0	0	16	0,35± 0,09	11	0,24± 0,07	27	0,58± 0,11
10^{-1}M $CdCl_2$	4540	17	0,37± 0,09	18	0,39± 0,09	43	0,95± 0,14	78	1,71± 0,19
Epitalon+10^{-1}M $NiCl_2$	4900	0	0	11	0,22± 0,07	13	0,27± 0,07	24	0,49± 0,09
10^{-1} M $NiCl_2$	4680	11	0,24± 0,07	19	0,39± 0,09	16	0,34± 0,08	46	0,97± 0,15
Epitalon	4720	0	0	2	0,04±0,02	1	0,02±0,02	3	0,06±0,03
control	5100	0	0	0	0	0	0	0	0

Our results may be explained by obtained data dealing with the mechanism of Epitalon activity. It is known, that Epitalon and other peptide compounds participate in gene expression and transspecific regulation of biosynthesis. They decrease pathological processes in cells such as DNA damage, mutations, malignant transformation and increase reparation process of cell homeostasis.

Epitalon also has strongly expressed antioxidant effect by activating antioxidant systems of cells [Khavinson V. K., et al., 2001].

As it follows from obtained data about the mechanism of heavy metals mutagenicity, Pb, Cd and Ni have direct and indirect mutagenic effects expressed in DNA damage [Chao Jui-L., Yang Jia-Ling, 2001; Fracasso M. E. et al. 2002], or in inhibition of excision reparation [Mouron S. A., et al. 2001], also oxidative stress [Brennan R. J., et al., 1996] and free radicals induction [Marzyna M. et al., 2002].

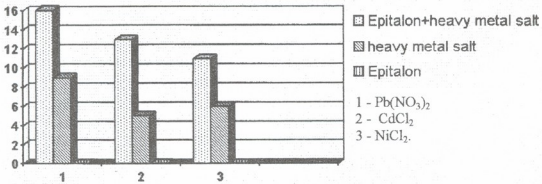

Fig. 1. The total number of sex-linked recessive lethal and sublethal mutations after treatment with single heavy metal salts and in combination with Epitalon

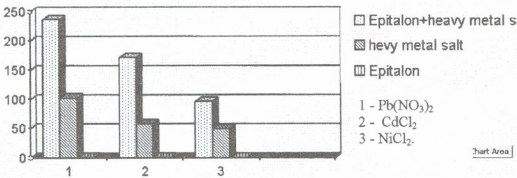

Fig. 2. The total number of wing morphoses after treatment with single hevy metal salts and in combination with Epitalon

Recently it is found, that Epitalon and other peptide bioregulators induce chromatin decondensation in human lymphocytes [Khavinson V. K., et al., 2003]. Therefore the DNA primary damages become more accessible for the reparation enzymes. Hence, it may be supposed, that Epitalon has the antimutagenic properties.

We have also studied the influence of Epitalon on some physiological activity (morphoses induction) caused by heavy metal salts. Three categories of wing morphoses - long-winged, single-wing-open and double-wing-open was accounted in Drosophila melanogaster after treatment with the high mutagenic doses (10^{-1}M) of $Pb(NO_3)_2$, $CdCl_2$ and $NiCl_2$ [Nibladze, Jmukhadze 2003]. These morphoses are phenotypicaly identified with the following known mutations - long-winged - Gull, single-wing-open – divergent and double-wing-open – warped.

After combinative treatment with Epitalon and above mentioned compounds the strongly decreased number of single-wing-open and double-wing-open morphoses was revealed in comparison to the effect of single heavy metal salts. As to the long-winged morphoses, they have not appeared, while heavy metal salts induce the most number of long-winged morphoses then other ones.

These results indicate, on the one hand the Epitalon modifying activity and, on the other hand, the specific character of wing–morphoses formation

Thus, in combination with heavy metal salts Epitalon strongly decreased the frequency of sex-linked recessive lethal and sublethal mutations induced by heavy metal salts. The modifying effect of Epitalon was confirmed by decreasing wing morphoses caused by heavy metal salts in *Drosophila melanogaster*. So, we supposed that Epitalon is found to have the antimutagenic properties.

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ეპიტალონის გავლენა მძიმე მეტალთა მარილებით გამოწვეულ მუტაბენურ ეფექტზე დროზოფილაში

ნიბლაძე ნ., ჯმუხაძე ნ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

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რეზიუმე

შესწავლილია ეპიტალონისა და მძიმე მეტალთა მარილების ($Pb(NO_3)_2$, $CdCl_2$ და $NiCl_2$) კომბინირებული მოქმედების გენეტიკური ეფექტი *Drosophila melanogaster*–ზე. ეპიტალონმა გამოავლინა მამოდიფიცირებელი მოქმედების მკვეთრად გამოხატული ხასიათი მძიმე მეტალთა მარილებით გამოწვეული სქესთან შეჭიდული რეცესიული ლეთალური და სუბლეთალური მუტაციების შემცირების გზით. მან შეამცირა აგრეთვე მძიმე მეტალთა მარილების მოქმედებით მიღებული ცალფრთაგაშლილი და ორფრთაგაშლილი ტიპის ფრთის მორფოზების რიცხვი, ხოლო გრძელფრთიანი მორფოზების წარმოქმნა მთლიანად დათრგუნა. აქედან გამომდინარე ჩვენ ვვარაუდობთ, რომ ეპიტალონს ახასიათებს ანტიმუტაგენური მოქმედების უნარი.

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Genetics

THE CYTOGENETIC EFFECT OF AMMONIUM AND LEAD NITRATES ON ALLIUM CEPA SEEDS OF DIFFERENT AGE

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Abstract

Single and combined cytogenetic effect of ammonium and lead nitrates (0,001M and 0,01M) has been studied in meristem tissue cells of roots sprouted from one- and five-year-old seeds of Allium cepa. The both doses of the biotested compounds were found to be mutagenic. The dose-dependent character of the cytogenetic effect was detected that was more apparent in the case of old seeds.

Key words: Allium cepa, biotesting, heavy metal, nitrate, chromosomal aberrations.

Introduction

The biosphere is inhabited by the organisms of different species and age. They all expose to complex influence of various pollutants. Among the pollutants special attention should be paid to the chemical compounds used in agriculture and industry. Some of them are characterized by genetic, toxic, teratogenic and carcinogenic activities [Anard at all., 1997].

Nitric compounds are widely used in order to improve the soil fertility. They are of great importance for favouring plant growth and harvest increase. Although, accumulation of high doses in soil may have negative results [Keve Koroles at all., 1996].

In industry and military operations , as well as in transport facilities the compounds of lead are used. This metal also accumulates in soil and water and has only harmful influence on living organisms [Sengupta, Ghosh, 1995].

According to such uncontrolled accumulation of nitric and lead compounds in biosphere they can alter genetic system of living organisms towards undesirable direction.

Proceeding from that, we studied the cytogenetic effect of single and combined activities in one- and five- year-old seeds of Allium cepa.

Material and methods

Biotesting of lead and ammonium nitrates has been performed using one- and five-year-old seeds of Allium cepa. 0,001M and 0,01M distilled water solutions of ammonium and lead nitrates were used. The distilled water was used for germination of control samples.

The microscopic analysis revealed different types of cytogenetic damages in meristem tissue cells of roots.

By cytogenetic method the following chromosome aberrations were registered – anaphase and telophase bridges, chromosome rings and fragments, chromosome fusion, heteroploidy and asymmetric anaphases. The mitotic indices were estimated for each sample.

Results and discussion

The high doses of ammonium and lead nitrates are more affective at the onset of germination that should be attributed to the stimulating activity of high doses of nitrogen on growth capacities. At the next stage the high doses of lead and nitrate induced slowing of growth processes and in five-year- old seeds root growth is even stopped .

The microscopic analysis of meristem cell showed that the both doses of ammonium and lead nitrates caused definite cytogenetic aberrations and other rearrangements varied within the range of 8,09% - 23,99 %, but in the case of lead nitrate from 18,14% to 30,36 %, that reliable exceeded the spontaneous level of aberrations in control samples (2,03 %). Thus, it was shown higher genotoxic activity of lead nitrate with the prevalence of bridge- and fragment- containing anaphases.

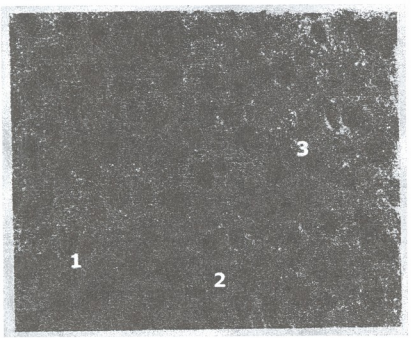

Fig.1. Microphotograph - Chromosomal aberrations in meristem tissue cells of roots
1- Anaphase bridge, 2- Anaphase fragments, 3- Asymmetric anaphases.

The mitotic activity of meristem tissue cells was low in the roots of five-year-old onion seeds, but spontaneous level of chromosome aberrations was higher in comparison with one-year- old seeds (6,76 %). In the five- year –old seeds the lead nitrate in the dose of 0,01M induced the complete inhibition of mitosis - the cells were stopped at the stage of interphase and the nuclei were vacuolated. It was obvious that the high dose of lead induced irreversible fusion of chromatin and the cells became unable to enter the next stage of mitosis.

In case of combined activity of ammonium and lead nitrates their dose –dependent effect has been better exhibited and in case of high doses the frequency of chromosome aberrations were equal to 37,21% in one-year-old seeds and to 47,44 % in five-year-old seeds.

Among the chromosome disorders in case of combined activities of the compounds the most frequent were the fused metaphases (Fig. 1. microphotograph demonstrates the chromosomal aberrations).

Thus, the both examined compounds are revealed to be the mutagenic agents. They have the dose-dependent cytogenetic activities increasing in parallel to the age of the seeds.

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Allium cepa –ს სხვადასხვა ასაკის თესლებზე ამონიუმისა და ტყვიის ნიტრატის ზემოქმედების ციტოგენეტიკური ეფექტი

შევარდნაძე გ., გოგინაშვილი ქ., სულაბერიძე ს.

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(მიღებულია 10.05. 2004)

რეზიუმე

შესწავლილია ამონიუმისა და ტყვიის ნიტრატის (0,001 M და 0,01M) განმხოლოებული და კომბინირებული ზემოქმედების ციტოგენეტიკური ეფექტი Allium cepa-ს ერთი და ხუთ წლიანი თესლების ღივის მერისტემული ქსოვილის უჯრედებში. ბიოტესტირებული ნაერთების ორივე დოზა წარმოადგენს მუტაგენურ აგენტებს. გამოვლინდა მათი დოზა-დამოკიდებული ციტოგენეტიკური ეფექტი, რაც იზრდება თესლის ასაკის გაზრდის ფონზე.

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FREQUENCY OF CHROMOSOME ABERRATIONS AND CHARACTERISTICS OF RIBOSOMAL CISTRONS ACTIVITY IN PATIENTS WITH SENILE DEMENTIA

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Abstract

The lymphocyte cultures derived from 10 individuals (age 70-80) with sporadic senile form of dementia have been investigated. The following parameters have been evaluated: spontaneous levels of structural chromosomal disorders and transcriptional activity of ribosomal genes. Significantly increased level of chromosome instability in demented patients compared with control group (age 70-80) was revealed. The study of nucleolar organizing regions showed, that the frequency of Ag-positive acrocentrics and the indices and types (DD, DG, GG) of acrocentric chromosome associations in all cases corresponded to the mentioned parameters in elderly controls and were less than in young controls.

Key words: senile dementias, chromosome aberrations, acrocentric chromosomes, nucleolar organizing regions (NORs).

Introduction

Since the discovery of inherited dementias, their cytogenetic investigations have been intensively started [Trippi et al., 2001, Hedera et al., 2002]. But in spite of the existence of numerous scientific data, it is not possible to distinguish one approach or direction from the other because of their opposite character. The results of various cytogenetic experiments differ from each other within the vast range. Therefore the additional researches are necessary for the reason to explain the existed fluctuations [da Silva et al., 2000, Clarck et al., 2003]. The main goal of our research was to study genetic bases of dementias' clinical picture using different cytogenetic test-systems.

Material and Methods

Cytogenetic investigations were carried out on the phytohemagglutinin (PHA) stimulated peripheral blood lymphocytes of demented patients (diagnosed in National Institute of Neurology of Georgia) and healthy donors (I control group - 70-80 years old, II control group - 20-30 years old). Cultivation of lymphocytes, preparation and staining of chromosome slides were performed by Moorhead's standard method. The frequency of structural chromosomal disorders was assessed in 861 metaphases of 10 individuals (age 70-80) affected with senile dementia. About 60-100

metaphases for each patient were analyzed. Structural chromosomal aberrations were defined by International System of Cytogenetic Nomenclature (ISCN, 1985).

The level of transcriptional activity of ribosomal genes was assessed according to the incidence of cells with acrocentric chromosome associations, the number of nucleolus organizer regions (NORs) in all acrocentrics and the intensity of Ag-staining. The sizes of silvered segments were evaluated by 2-point scale system: 1-small segments (less than chromatid width); 2-large (equal to or more than chromatid width). The following parameters have been determined in order to assess the frequency of acrocentric chromosome associations: number of association containing cells; an average number of associations per cell; number of chromosomes participating in the associations and the frequency of "opend" associations [Lezhava, 2001].

Results and Discussion

861 metaphases of 10 individuals affected with senile dementia have been analyzed in order to determine the spontaneous level of structural chromosomal disorders. Noticeably, the variability of aberrant metaphases as well as chromosome disorders were revealed within the individuals. The frequency of aberrant cells varied among patients within the range of 2% to 12%, significantly increased level of such cells was detected in 7 individuals. As for the frequency index of aberrant cells, no differences were revealed among the patients with various forms of dementia that gave us the opportunity to calculate average number of abnormal cells for 10 individuals and it was equal to 8,94±1,1%. This index is significantly higher than the corresponding values in both control groups (I control - 2,8±0.52%; II control-1,6±0,4%. Fig. 1). The analyses of aberration types showed that common chromosome damages were single and pair fragments. It is important that cells with multiple aberrations were also registered.

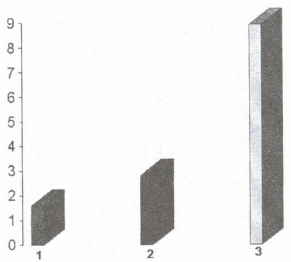

Fig.1. Frequency of structural chromosome disorders in (1)- control group (20-30 yrs); (2)-control group (70-80 yrs) and (3) individuals with senile dementia

These results are in agreement with the data indicating increased level of chromosome instability for various forms of dementia. Cytogenetic research of Alzheimer's disease (AD) showed that decreased activity of DNA-repair system takes place [Bradley et al., 1989], the result of which is increased frequency of chromosomal aberrations in case of these pathologics [Trippi et al., 2001]. Thus, in scientific literature the opposite results exist as well [White et al., 1981]. They suggest no increase in chromosome abnormalities in AD patients.

As for the frequency of Ag-positive NORs and their distribution through the acrocentric chromosomes, a mean value of these indices (5,36 Ag^{2+}-NOR per cell) was not statistically different from control values (5,30 and 5,23 respectively). A mean percentage of satellite association containing cells (44,7±2,94%) was within the same range as compared with elderly controls (44,6±4,52) and was less than the value of young control group (51,56±2,3) (Fig. 2).

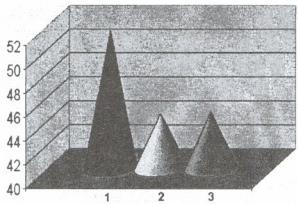

Fig. 2. Frequency of acrocentric chromosome associations in (1) young controls; (2) elderly controls and (3) demented patients

These results are in agreement with the data [Lu et al., 1998] about nucleolar organizer regions of hippocampal neurons in AD. It was shown that the frequency of Ag-NORs decreased in the elderly and the AD groups in comparison with the young group. However, the area of stain and the integrating absorption of the nucleoli of the hippocampal neurons relatively increased in the AD group as compared with the elderly group indicating to the strengthened genetic expression of the cell's population in AD patients. Payao et al., also suggest that the decline of transcriptional activity of NORs takes place in demented individuals [Payao et al., 1994].

Consequently, we suppose that the mentioned results can be induced by chromatin modification engendering ribosomal cistron inactivation in case of *in vivo* and *in vitro* ageing, premature ageing [Lezhava et al., 1992, Lezhava, 1999] and also in Down's syndrome [Saadat et al., 2000]. The factor of chromatin modification might be foreseen in the selection procedure of remedies in order to cure such a harmful pathologies [Lezhava et al., 2004].

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ქრომოსომათა სტრუქტურული დარღვევების სიხშირე და რიბოსომული ცისტრონების აქტივობის მახასიათებლები სენილური დემენციების დროს.

ტაბატაძე ნ., დადუნაშვილი ე., ამუნაშვილი ნ.

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(მიღებულია 10.05.2004)

რეზიუმე

ქრომოსომათა სტრუქტურული დარღვევების სპონტანური დონისა და რიბოსომული გენების ტრანსკრიპციული აქტივობის განსაზღვრის მიზნით შესწავლილია სენილური დემენციის სპორადიული ფორმით დაავადებული 10 ინდივიდის (ასაკი 70-80წ) ქსოვილოვანი კულტურები. გამოკვლევებმა აჩვენა, რომ ადგილი აქვს ქრომოსომების სტრუქტურული დარღვევების მნიშვნელოვან მომატებას დემენციით დაავადებულ ინდივიდებში საკონტროლო (70-80წ) ჯგუფთან შედარებით. ბირთვაკ-მაორგანიზებელი უბნების შესწავლამ კი აჩვენა, რომ Ag-პოზიტიური უბნებისა და აკროცენტრულ ქრომოსომათა ასოციაციების სიხშირის მაჩვენებლები და ტიპები (DD, DG, GG) ყველა შემთხვევაში შეესაბამება საკონტროლოდ აღებულ მოხუც ინდივიდთა აღნიშნულ მაჩვენებლებს და ჩამორჩება ახალგაზრდებისას.

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PATTERNS OF THE EXPRESSION OF CD32 AND CD64 ON MONOCYTES OF PATIENTS WITH B CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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Abstract

Character of phagocytosis mediated by monocytic Fcγ Receptor I (CD64) and Fcγ Receptor II (CD32) in patients with B cell chronic lymphocytic leukaemia (B-CLL) may explain increased incidence of bacterial and viral infections in B-CLL. Patterns of CD32 and CD64 expression on monocytes of treated and untreated B-CLL patients and patients at the disease stage Rai 0 – 4 have been explored. It has been revealed that treatment lowers the expression of CD64 on monocytes, and the expression of CD64 decreases with the increase of disease stage.

Key words: B cell chronic lymphocytic leukaemia (B-CLL), CD32, CD64, monocytes.

Introduction

B cell chronic lymphocytic leukaemia (B-CLL) is a progressive accumulation of functionally incompetent, long living B lymphocytes in peripheral blood and bone marrow. The course of the disease is variable, however the majority of patients survive for many years with little or no treatment and gradually increasing number of B-CLL cells in circulation [Jewell, 1999].

Phagocytic Fcγ receptors (FcγR) bind to the Fc portion of IgG-opsonized pathogen and cross-linking of these receptors on the phagocyte eventually leads to the phagocytosis of the agent [Fossati et al., 2001].

CD32 expressed on monocytes, granulocytes, platelets and B and T lymphocytes belongs to the FcγRII class family and consists of intracellular, transmembrane and extracellular domains. Its density is similar on monocyte/macrophages. CD32 is relatively stable towards cytokine action, whereas GM-CSF increases the density of monocytic form and IL-4 supresses the receptor expression [Ravetch et al., 1991; Kruger et al., 1997].

CD64, apart from other cell types, is also expressed on monocytes, macrophages and dendritic cells. It consists of cytoplasmic, transmembrane and extracellular domains and belongs to the FcγRI class family. The density of CD64 on freshly isolated granulocytes is low, but IFNγ

increases its density. IL-10 acts in a similar way, whereas IL-4 and IL-13 diminish the density of CD64 [Ravetch et al., 1991; Ohsaka et al., 1994; Wang et al., 2001].

The expression character of CD64 on monocytes and neutrophils of B-CLL patients has been previously investigated by us and the purpose of the present study was to explore the pattern of CD32 and CD64 expression on monocytes of the same target group taking into account the disease stage and treatment mode.

Materials and methods

We studied 15 B-CLL patients at the disease stage Rai 0, 1, 2, 3 and 4 and under different treatment schemes, who were previously diagnosed with B-CLL using standard clinical criteria. Patients were from the Mukhadze Institute of Haematology and Blood Transfusiology. Diagnosis for these patients was confirmed by flow cytometric analysis of CD5+CD19+ phenotype.

Peripheral blood mononuclear cells (PBMC) from heparinized blood were separated by centrifugation on Ficoll-Hypaque density (1,077g/l) gradient (Sigma). After the separation cells were washed twice with Phospate-Buffered Saline (PBS). Cells were stained with FITC-conjugated monoclonal antibodies (mAb) anti-CD32 or anti-CD64 and IgG1 isotype control (all – Pharmingen) for 45 min at 4°C, washed twice in PBS and fixed with 1% paraformaldehyde. The samples were analyzed using FACScan flow cytometer (Becton&Dickinson). Monoclonal antibodies for CD5+CD19+ phenotype analyses included FITC-conjugated anti-CD5 and anti-CD19 (both - Immunotech).

Data were analyzed, gating on monocytes in the SSC-FSC dot plot. Within this gate markers were set on the isotype control to define the negative population (Figure 1). Data were expressed as histograms or mean fluorescence intensity (MFI). For each sample percentages of monocytes expressing CD32 and CD64 and MFI of CD32+ and CD64+ were measured.

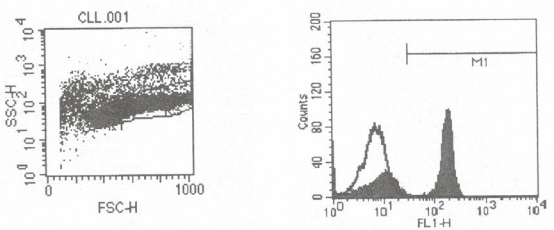

Fig. 1. Immunophenotyping of monocytes using flow cytometry: a) distribution of cells according to their size and granularity. R3 population is a population of the viable monocytes; b) representation of monocyte immunophenotyping as a histogram. An isotype control is shown as a thick contour.

The data were statistically analyzed using the Mann-Whitney non-parametrical test. Given values represent average (M) and its standard deviation (SD).

Results and discussion

The disease stages 0-4 of B-CLL patients was determined according to the modified Rai criteria [Rai , 1987]. Patients were subdivided into two groups – those at the earlier stages 0 and 1 and those at the later disease stages from 2 to 4.

From the patients studied half were untreated and the rest were treated with steroids (Prednisone), Leucerine, COP (Cyclophosphamide, Oncovin, Prednisone) or ACOP (Adriablastin, Cyclophosphamide, Oncovin, Prednisone).

Monocytes of B-CLL patients revealed heterogeneous expression of CD32 and CD64 based on the disease stage and treatment character measured by the percentages of positive cells (Figures 2, 4) and MFI (Figures 3 and 5).

As it is shown in Figures 2 and 3, there was no appreciable difference in the expression of CD32 between the disease stages measured either by the percentages (91.77±2.52% for stages 0-1 and 85.59±14.36% for stages 2-4), or the MFI (377.34±319.08 and 454.64±302.59 accordingly).

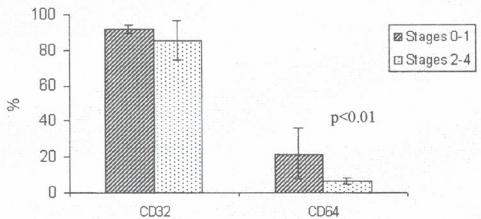

Fig. 2. Percentages of CD32+ and CD64+ monocytes from B-CLL patients at the different disease stages.

In case of CD64, both percentages and MFI decreased significantly with the disease stage (stages 0-1: 21.66±11%, stages 2-4: 6.54±0.12%, $p<0.01$; MFI for stages 0-1: 67.02±62.05, for stages 2-4: 21.27±12.36, $p<0.01$).

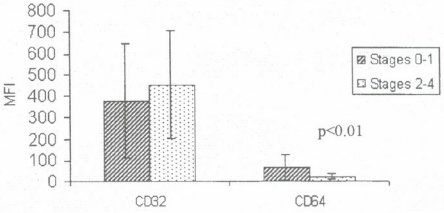

Fig. 3. Mean fluorescent intensity of CD32 and CD64 expression on monocytes of B-CLL patients at the different disease stages

We have also shown that the treatment affects expression of CD64: the treatment decreased the receptor expression on monocytes (16.60±13.25% for untreated patients and 8.58±9.42% for treated, $p<0.05$; MFI – 72.86±51.44 and 17.40±11.42 accordingly, $p<0.01$) (Figures 4 and 5). Expression of CD32 did not change after treatment.

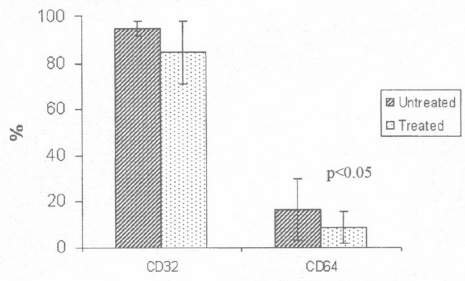

Fig. 4. Percentages of CD32+ and CD64+ monocytes from untreated and treated B-CLL patients

It is obvious that the expression of CD32 on monocytes of B-CLL patients is stable, whilst CD64 is a subject of modulation. Revealed pattern of CD64 expression on monocytes of B-CLL patients may be due to IL-4 action, which reduces number of CD64+ monocytes and lowers receptor density with the increase of the disease stage. It is well known that B-CLL cells produce

IL-4 which can downregulate the expression of CD64 on monocytes. We have shown previously that monocytes in B-CLL patients are generally characterized by the impaired expression of CD64 [Akhobadze et al, 2003]. It seems now that CD64 is further gradually downregulated on monocytes through the course of B-CLL, which may be due to a permanent release of IL-4 by B-CLL leukaemic cells.

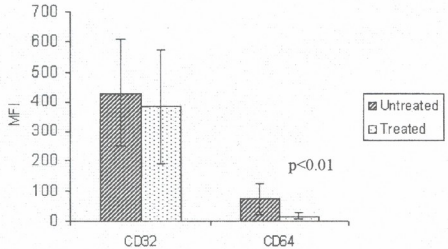

Fig. 5. Mean fluorescent intensity of CD32 and CD64 expression on monocytes of untreated and treated B-CLL patients

FcγRI and FcγRII on the surface of monocytes mediate attachment and entry of microorganisms opsonized with immunoglobulins into cells. Ligand binding to the FcγR induces the release of TNF and triggers the respiratory burst. Thus, it can be concluded that decreased number of CD64+ monocytes together with its low density can be one of the factors leading to B-CLL patient susceptibility to infectious complications. The patients at the more advanced stages of the disease have lower expression of CD64 and as a result impaired phagocytic activity of monocytes. Complex cytotoxic drugs (Prednisolone, Leucerine, Cyclophosphamide) used for the treatment of B-CLL patients cause further reduction of CD64 expression on monocytes of the treated patients. The treatment, therefore can contribute to the susceptibility of B-CLL patients to infectious diseases. This should be taken into consideration while administering particular treatment schemes.

Acknowledgement

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B-ქრონიკული ლიმფოციტური ლეიკემიით დავადებულ პირების მონოციტებზე CD32 და CD64 მარკერების ექსპრესიის თავისებურებანი

ახობაძე თ.[1], ჟორდანია ც.[1], კულიკოვა ნ.[1], ქარდავა ლ.[1], გაჩეჩილაძე ნ.[1], ღირდალაძე დ.[2], იოსავა გ.[2], ფორაქიშვილი ნ. [1]

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(მიღებულია 14.04.2004)

რეზიუმე

B-უჯრედული ქრონიკული ლიმფოციტური ლეიკემიის (B-CLL) მქონე პირების მონოციტების Fcγ რეცეპტორ I (CD64) და Fcγ რეცეპტორ II (CD32)-ით განპირობებული ფაგოციტოზის ხასიათმა შეიძლება ახსნას ბაქტერიული და ვირუსული ინფექციების სიხშირის მატების მიზეზი აღნიშნული დაავადების მქონე პირებში. ჩვენს მიერ გამოკვლეულ იქნა B-CLL-ის მქონე ნამკურნალევ და არანამკურნალევ და დაავადების Rai 0-დან მე-4 სტადიის მქონე პირების მონოციტებზე CD32 და CD64-ის ექსპრესია. აღმოჩნდა, რომ მკურნალობა აქვეითებს CD64-ის პროცენტულ მაჩვენებელს, და რომ CD64-ის ექსპრესია მონოციტებზე მკვეთრად კლებულობს დაავადების სტადიის მატებასთან ერთად.

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Immunology

THE ROLE OF COMPLEMENT IN THE CLEARANCE OF HUMAN CHORIONIC GONADOTROPIN (HCG) CONTAINING IMMUNE COMPLEXES

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Abstract

The β-chain of a recombinant molecule of human chorionic gonadotropin (hCG) containing a single point mutation of an arginine to glutamic acid at a position 68 [hCGβ(R68E)] is a potent candidate for anti-fertility or/and anti-tumor vaccine development. However, the immune complexes (ICs) formed after vaccination must be cleared by the phagocytic system prior to the interaction between hCG and its specific receptor to prevent the physiological effect of hCG. In addition the ICs can induce various pathologies. It is reported that complement may play an important role in opsonization and clearance of ICs formed as the result of hCGβ(R68E) immunization.

Key words: human chorionic gonadotropin (hCG), immune complexes (ICs), complement.

Introduction

Immunological processes are of major importance in reproduction. Immune reactions take part in gestation as well as in preventing pregnancy. Therefore immunocontraception or fertility control due to vaccines is the current strategy for family planning. The rationale of these fertility-control vaccines is to induce hormonal or cell-mediated immunity against either a hormone or gamete antigens important for reproduction. A series of hormones are involved in a cascade fashion in regulating reproductive processes, and fertility can be disrupted by eliciting an immune response against virtually any component of the cascade process including human chorionic gonadotropin (hCG) [Talwar et al., 1994]. hCG is produced by early blastocysts and later by trophoblastic cells of placenta and is responsible for the formation of *corpus luteum* that is the source of progesterone. hCG is the earliest marker of pregnancy and is detectable in serum of pregnant woman at about 6-7 days after fertilization (Iles and Chard, 1993). Normal production of hCG is vital to successful reproduction in humans. An advantage in choosing hCG as a target for immunocontraception is that its inactivation prevents pregnancy but would not interfere with other female physiological processes, such as ovulation and production of steroid sex hormones (Chikadze et al., 2003).

hCG is a heterodimeric molecule consisting of an α-chain, common to all members of the glycopropein hormone family, non-covalently associated with a β-chain unique to each hormone. Both the α- and β-chains are composed of three loops held in place by a cystine knot of the disulfide bounds, a structural motif also found in transforming growth factor β (TNF-β), neuronal

growth factor (NGF), platelet-derived growth factor β (PDGF-β) and various other growth hormones (Lund and Delves, 1998).

Currently it was shown that the native glycoprotein and its subunits are synthesized by malignant cells of both gonadal and non-gonadal origin. It is believed that hCG plays a role of autocrine growth factor for tumor cells (Butler et al., 1999). Clinical studies showed that the anti-hCG response plays an important role in life-saving of patients with tumors. Thus hCG as a tumor-associated marker represents a candidate target protein for active immunotherapy (Geissler et al., 1997).

To eliminate the cross-reactivity between the epitopes of the β-chains of hCG and luteinizing hormone (hLH) a number of different hCGβ-chain mutants have been generated at the University College London, UK, one of which, hCGβ(R68E), is capable of stimulating a strong antibody response towards native hCG but not hLH. The mutant sera has been generated in experimental rabbits. It was shown that the mutant antibodies were mainly focused upon the carboxiterminal region of the β-chain [Porakishvili et al., 2002]

This vaccine can be used for both immunocontraception and anti-tumor treatment. The remaining problem, however, is associated with the immune complexes (ICs) formed after vaccination. Since the receptor-binding site of hCG within the IC remains free, the formation of ICs will not necessarily prevent hCG from binding to the receptor and will not block its function unless effectively cleared from circulation by phagocytes [Porakishvili et al., 2002]. In addition circulating ICs can induce various pathologies including autoimmune diseases.

Our previous experiments indicated that hCG-containing ICs are successfully engulfed by monocytes and neutrophils, although the mechanisms of phagocytosis have not been yet clarified [Chikadze et al., 2003]. The aim of our current research is to study the involvement of complement in the phagocytosis of ICs containing native molecules of hCG and mutant serum, obtained from the rabbits immunized with hCGβ(R68E).

Materials and Methods

Formation of immune complexes

Native hCGαβ heterodimer purified from human pregnancy urine (Zimed, USA) was labeled with fluorescent isothiocyanate (FITC, Sigma). Immune complexes were formed by FITC-conjugated hCG and mutant anti-hCGβ(R68E) rabbit serum diluted in PBS by 25. The formation of ICs was achieved by incubation of hCG and the sera at 37°C for 2 hours and then at 4°C for 24 hours for IC precipitation. In separate experiments mutant serum was heated to 56°C for inactivation of complement and ICs were formed as described above. hCG only with PBS instead of the immune sera was used as a control.

Phagocytosis of immune complexes by blood monocytes and neutrophils

Peripheral blood mononuclear cells (PBMC) and polymorphonuclear neutrophils (PMN) were separated by centrifugation in Ficoll-Hypaque (Sigma) gradient (1.077 and 1.119 respectively) from heparinized blood of 7 healthy donors. After the separation cells were washed twice with phosphate-buffer saline (PBS, Sigma) and the concentration was adjusted to 1million in 1ml.

The mixture of ICs or the control hCG and the cells were incubated at 37°C for an hour in test-tubes. The amount of FITC-positive phagocytic cells were measured by flow cytometry (Becton&Dickinson) by gating on monocytic and neutrophil populations.

Precipitation of immune complexes

3, 4, and 10% polyethylenglycol (PEG, Sigma) solutions were used to precipitate ICs with high, middle and low molecular weights respectively. The mixtures of PEG and ICs were incubated at room temperature for 2 hours. Flat-bottomed microtiter strips (EFLAB) were filled with the mixture and then read at A450 in a spectrophotometer (Humanreader).

Statistical analysis

The validity of the results was determined according to the Student t-test. The values on the charts represent an average and a standard deviation.

Results and Discussion

The results show that the largest precipitates of ICs formed by the native hCG and the mutant sera were obtained with the concentration of PEG 3% and 10% that mainly large and small sized immune complexes were present (Figure 1).

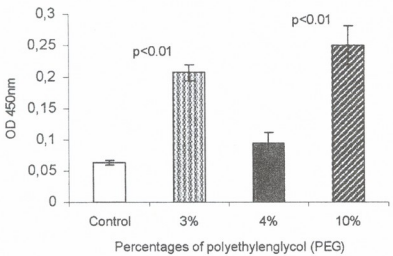

Fig. 1. Precipitation of immune complexes in PEG

The phagocytic cells – monocytes and neutrophils - carry the surface receptors for IgG (Fcγ-receptors, FcγR) and for the complement components C3b (complement receptor type 1, CR1) and iC3b (CR3). Within certain size limitations, any material covered by these opsonins will be ingested including simple ICs containing only one particular antigen [Roitt and Delves, 1992]. Since the leading hCG-containing ICs are small-sized, we suppose that they incorporate complement components which would mediate the phagocytosis by both monocytes and neutrophils. The fact that the large-sized ICs were also present in the precipitate may indicate that

complement-containing small ICs can also form large conglomerates which are usually favored by phagocytic cells.

To confirm the involvement of complement in phagocytosis of the native hCG/mutant sera-containing ICs we studied the percentages of monocytes and neutrophils engulfing the ICs with FITC-conjugated hCG. The IC-forming sera was either used intact (positive control) or pre-heated at 56° C to inactivate the complement. The negative control contained hCG molecules only with PBS instead of antibodies (Figure 2).

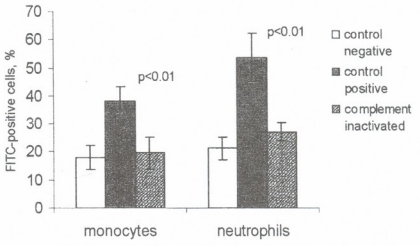

Fig. 2. Phagocytosis of ICs containing complement-inactivated or intact sera by normal blood monocytes and neutrophils

Our data shows that the percentages of FITC-positive monocytes and neutrophils when the complement-inactivated IC samples were used are significantly ($p<0.01$) decreased compared with the positive controls almost to the basic (hCG only, no ICs) level. Such results were obtained in six out of seven donors for monocytes and in five out of seven donors for neutrophils. Since the inactivation of complement abrogates phagocytosis, we can assume that the receptors to C3b are involved in the process of engulfing.

Our study therefore demonstrated that complement participates in opsonization of the immune complexes formed by native hCG and rabbit sera obtained by immunization with the mutant hCGβ(R68E). As a result small and large-sized ICs are created. These are readily phagocytosed by both blood monocytes and neutrophils via the CR1 and CR3-receptors. The data indicate that the immune complexes formed by native hCG and mutant hCGβ(R68E) sera can be successfully cleared by the phagocytic system from the circulation and that this process is mediated by complement. This further argues for a high quality and effectiveness of contraceptive and anti-tumor vaccine based on hCGβ(R68E).

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კომპლემენტის როლი ადამიანის ქორიონული გონადოტროპინის (აქგ) შემცველი იმუნური კომპლექსებისაგან ორგანიზმის გაწმენდაში

ჯანიკაშვილი ნ., ჭიკაძე ნ., გაბუნია ხ., ბურჯანაძე ლ., სერედა ლ., ამადლობელი ნ., გაჩეჩილაძე ნ., ფორაქიშვილი ნ.

იმუნოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 03.05.2004)

რეზიუმე

ადამიანის ქორიონული გონადოტროპინის (აქგ) β-ჯაჭვის რეკომბინანტული მოლეკულა [აქგβ(R68E)], რომლის ამინომჟავურ თანმიმდევრობაში წერტილოვანი მუტაციით 68-ე ადგილზე არგინინი ჩანაცვლებულია გლუტამინის მჟავით, წარმოადგენს ძლიერ კანდიდატს ანტიფერტილური და/ან ანტიისმინური ვაქცინების შესაქმნელად. ამავე დროს, ვაქცინაციის შედეგად წარმოქმნილი ჰორმონ-ანტისხეულის მოცირკულირე იმუნური კომპლექსები (მიკ) სწრაფად უნდა მოსცილდეს ცირკულაციას ფაგოციტური სისტემის საშუალებით, რათა ხელი შეეშალოს აქგ ფიზიოლოგიური ფუნქციის განხორციელებას. გარდა ამისა, მიკ-მა შეიძლება გამოიწვიოს მრავალი პათოლოგია.

გამოვლენილია კომპლემენტის სისტემის მნიშვნელოვანი როლი აქგ β(R68E) რეკომბინანტული მოლეკულით იმუნიზაციის შედეგად წარმოქმნილი იმუნური კომპლექსების ოპსონიზაციასა და ორგანიზმიდან ელიმინაციაში.

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EFFECT OF CARBON SOURCE ON POLYSACCHARIDE METABOLISM OF *STREPTOMYCES VIOLACEUS*

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Abstract

Effect of variable carbon source (glucose, saccharose) on the intensity and development of polysaccharide synthesis in nutrient medium has been studied. It has been established that carbon source has an influence on growth dynamics of *Streptomyces violaceus*. During the growth in glucose nutrient medium the duration of the phases was: logarithmic phase – 0-24 h, exponential phase – 48-72 h, stationary phase – 72-96 h, a phase of dying – 100-124 h, while in saccharose nutrient medium – 0-48 h, 48-96 h, 100-120 h, 120-144 h, correspondingly. The change of carbon source in the nutrient medium had an influence on quantitative content of polysaccharides in cell wall of *Streptomyces violaceus* as well as on the intensity of exopolysaccharides in culture fluid. With the accumulation of *Streptomyces violaceus* biomass in glucose containing nutrient medium the intensification of cell wall and polysaccharide synthesis is increased compared with saccharose nutrient medium. A definite correlation between these processes was observed.

Key words: polysaccharide, cell wall, biomass, nutrient medium, culture fluid

Introduction

Recently an interest to the microorganisms polysaccharide has increased due to their use in clinical medicine [Petrovskaia, 2002]. It was found that these polymers are characterized by anticancerogenic features. Polysaccharides of actinomycetes appeared to be of interest in this aspect. Besides, monosaccharide content of polysaccharide may be used as a chemotaxonomic parameter in the systematization of microorganisms.

The goal of this investigation was to study peculiarities of *Streptomyces violaceus* polysaccharide (cell wall polysaccharides, neutral polysaccharides, exopolysaccharides) under conditions of variable carbon source in the environment.

Material and methods

Streptomyces violaceus obtained from the collection of microorganisms of Ketskhoveli Institute of Botany, Georgian Academy of Sciences was used for the investigation.

Actynomycetes were grown on Krasilnikov synthetic medium: $CaCO_3$ – 1 g, KNO_3 – 1 g, $MgSO_4$ – 0,5 g, K_2HPO4 – 0,5 g, NaCl – 0,5 g, $FeSO_4$ – a trace, H_2O – 1,0 l. Glucose and saccharose were used as carbon sources [Gauze et al., 1983, Krasilnikov, 1960].

The isolation of cell wall was performed according to R. L. Robson's method [Sotnikova, 2002], neutral polysaccharides were obtained using the method Zakharova and Kosenko [Zakharova and Kosenko, 1982]. Exopolysaccharides were precipitated from culture fluid using 5 valum of ethanol with the subsequent hydrolysis in 10N H_2SO_4 [Gerkhard, 1984]. For qualitative analysis of polysaccharides Zaitseva's method was applied [Nazarenko, 2001]

The results

The growth dynamics of *Streptomyces violaceus* has been studied in the cultivated medium under conditions of variable carbon source (glucose, saccharose). The results of our experiments are given on Fig. 1.

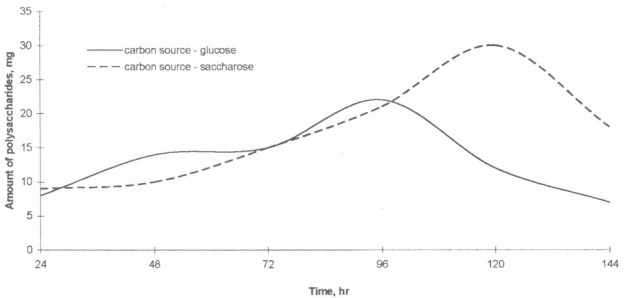

Fig. 1. Growth dynamics of *Streptomyces violaceus*

The analysis of the experiments has shown that cultivation period of *Streptomyces violaceus* during growth in glucose nutrient medium is 0-120 h. The duration of the phases is the following: logarithmic phase – 0-24 h, exponential phase – 48-72 h, stationary phase – 72-96 h, phase of dying – 100-124 h. The replacement of carbon source - glucose with saccharose induced changes in phase growth, particularly: 0-48 h, 48-96 h, 100-120 h, 120-144 h, correspondingly. The cultivated period lasts for 130-144 h. The usage of glucose as carbon source stimulates the growth of biomass.

Quantitative change of cell wall and effect of variable carbon source (glucose, saccharose) on cell wall mass according to the growth dynamics of *Streptomyces violaceus* have been investigated (Table 1).

Table 1. Cell wall mass of *Streptomyces violaceus* under conditions of variable carbon source

Growth phases of the culture	Cell wall mass in the biomass, mg/g	
	Carbon source - glucose	Carbon source - saccharose
Logarithmic phase	130,57	87,66
Exponential phase	100,67	80,345
Stationary phase	90,44	55,100
Phase of dying	112,52	57,75

As it is seen from the Table 1, cell wall mass changes in the process of culture growth: it is maximal in logarithmic phase and minimal – in stationary phase. Carbon source metabolism affects the quantitative outcome of cell wall. In case of glucose usage as carbon source cell wall mass of *Streptomyces violaceus* varies within the limits of 9-13% as compared to cellular mass. The usage of saccharose as carbon source cell wall mass reduces to 5,5-8,7%, what is clearly shown on Fig. 2.

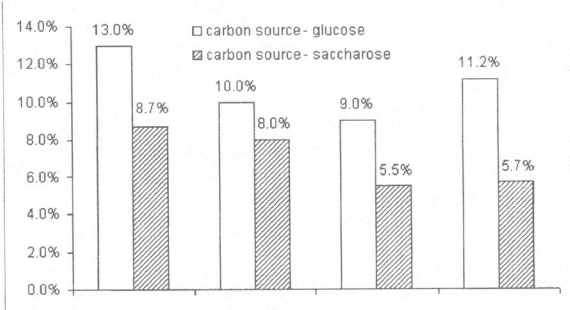

Fig. 2. Percentage changes in polysaccharides of *Streptomyces violaceus* cell wall according to growth phases under conditions of variable carbon source

Different carbon sources in the nutrient medium have an influence on the peculiarities of polysaccharide synthesis of *Streptomyces violaceus*, particularly on the intensity of cell wall polysaccharides (CPS), neutral polysaccharides (NPS) and exopolysaccharides (EPS).

As it is seen from the Fig. 3 and 4, qualitative growth of polysaccharides begins in logarithmic phase of culture growth and reaches its maximum in stationary phase. Total polysaccharides in cell wall, including the intensity of neutral polysaccharide synthesis and production of exopolysaccharides in culture fluid are minimal.

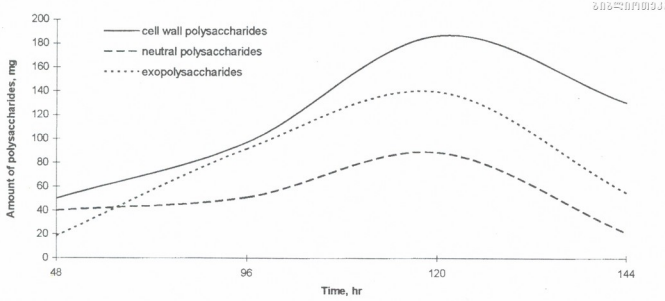

Fig. 3. The intensity of *Streptomyces violaceus* polysaccharide synthesis in glucose nutrient medium

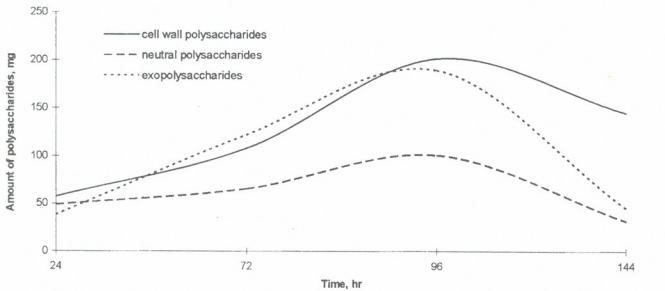

Fig. 4. The intensity of *Streptomyces violaceus* polysaccharide synthesis in saccharose nutrient medium

The amount (in %) of polysaccharide polymers of *Streptomyces violaceus* is shown on Fig. 5 and 6. During the whole period of culture development the amount of CPS varies within 5,7-20,1% (carbon source – glucose) and 5-19,6% (carbon source – saccharose). The amount of NPS synthesis in glucose nutrient medium is in the range 3,1-10,1% and in saccharose nutrient medium - 2,1-8,8%. The amount of EPS in culture fluid changes under conditions of different carbon sources is: in case of glucose - 3,8-18,9%, while in case of saccharose – 1,8-13,9%.

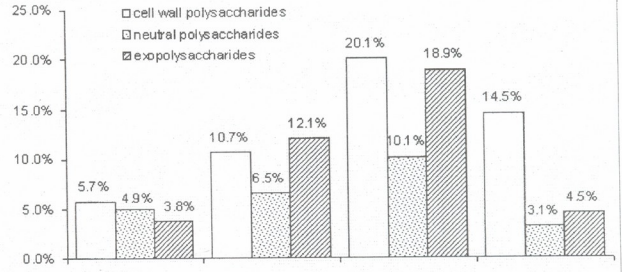

Fig. 5. Percentage indices of *Streptomyces violaceus* under conditions of carbon source – glucose

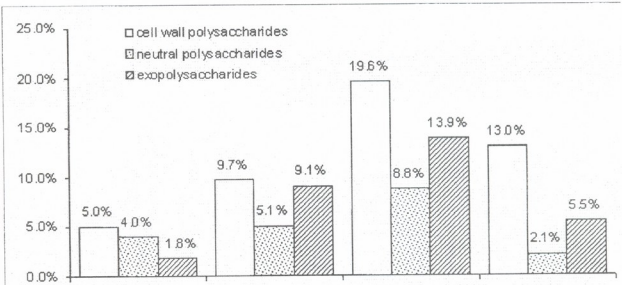

Fig. 6. Percentage indices of *Streptomyces violaceus* under conditions of carbon source – saccharose

Thin layer chromatography of studied polysaccharides has shown that variable carbon source has an influence on monosaccharide content of polysaccharides. The results are clearly shown in Table 2.

Table 2. Qualitative analysis of *Streptomyces violaceus* polysaccharides

Carbon source – glucose / Carbon source - saccharose			
Monosaccharides	CPS	NPS	EPS
Arabinose	+	+	+
Glucose	-	+	+
Fructose	+	-	-
Galactose	-	-	-
Ribose	+	-	-

So, the analysis of experimental data has shown that the usage of different carbon sources in the cultivated medium changes quantitative content of *Streptomyces violaceus* polysaccharides, at the same time it increases the duration of definite phases of culture growth development and does not change qualitative content of polysaccharides.

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ნახშირბადის წყაროს გავლენა *Streptomyces violaceus*–ის პოლისაქარიდულ ცვლაზე

კოტია ნ., ლომთათიძე ზ.

მიკრობიოლოგიისა და ვირუსოლოგიის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 03.05.2004)

რეზიუმე

შესწავლილია საკვებ არეში ნახშირბადის წყაროს (გლუკოზა, საქაროზა) ცვლის გავლენა *Streptomyces violaceus*–ის პოლისაქარიდების სინთეზის ინტენსივობასა და განვითარებაზე. დადგენილია, რომ ნახშირბადის წყარო გავლენას ახდენს *Streptomyces violaceus*–ის ზრდის დინამიკაზე. გლუკოზიან საკვებ არეზე ზრდისას ფაზათა ხანგრძლივობა შეადგენს: ლოგარითმული ფაზა – 0-24 სთ, ექსპონენციალური ფაზა – 48-72 სთ, სტაციონალური ფაზა – 72-96 სთ, კვდომის ფაზა – 100-124 სთ, ხოლო საქაროზიან საკვებ არეზე კი – 0-48 სთ, 48-96 სთ, 100-120 სთ, 120-144 სთ, შესაბამისად. საკვებ არეში ნახშირბადის წყაროს ცვლილება გავლენას ახდენს *Streptomyces violaceus*–ის უჯრედის კედლის და მის შემადგენლობაში შემავალი პოლისაქარიდების რაოდენობრივ შემადგენლობაზე. ასევე იცვლება კულტურალურ სითხეში ეკზოპოლისაქარიდების პროდუცირების ინტენსივობაც. გლუკოზიან საკვებ არეზე *Streptomyces violaceus*–ის ბიომასის ზრდასთან ერთად ადგილი აქვს უჯრედის კედლის და პოლისაქარიდების სინთეზის ინტენსიფიკაციას საქაროზიან საკვებ არესთან შედარებით. შეიმჩნევა გარკვეული კორელაცია ამ პროცესებს შორის.

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Microbiology

INFLUENCE OF SOME PHYSICOCHEMICAL FACTORS IN DIFFERENT MICROORGANISMS CAPABLE FOR DEGRADATION OF 2,4,6-TRINITROTOLUENE AND MINERAL OIL

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Abstract

The influence of some physicochemical factors (pH of cultivation medium, osmotic pressure, sources of carbon, nitrogen and phosphorus) on development of microorganisms characterized by high degradative ability of 2,4,6-Trinitrotoluene (TNT) and mineral oil has been studied. The results of experiments have showed that investigated microorganisms do not display exact speciality. They are capable to grow and degrade organic compounds (mineral oil or 2,4,6-trinitrotoluene or both of toxicants) at wide range of pH of cultivation media, osmotic pressure and also are able to apply different sources of carbon, nitrogen and phosphorus for growth; thus under natural environment probability of their existence and development of biodegradative capability is increased.

Key words: pH of cultivation medium, osmotic pressure, carbon, nitrogen and phosphorus sources

Introduction

Microorganisms effectively degrading different toxic compounds under laboratory conditions do not always display the capability in natural ecosystems. Various metabolic features of bacteria, determine their different survivability under natural conditions, having an influence on biodegradation process [M. Briglia et al., 1990; J. M. Middeldorp et al., 1990]. Non-selective conditions, which might be stressful, have great impact on growth efficiency of microorganisms and also on degradation of xenobiotics.

In connection with this, the goal of the present project is to study the influence of some physicochemical factors (pH of cultivation medium, osmotic pressure, sources of carbon, nitrogen and phosphorus) factors on developing of microorganisms characterized by high degradative ability of 2,4,6-Trinitrotoluene (TNT) or mineral oil.

Material and Methods

The objects of the research served microorganisms capable for intensive growth on media containing mineral oil and TNT; the cultures were isolated from soils of Georgia, contaminated with organic toxicants [N.Gagelidze et al., 2002].

Czapek's medium, beef-extract agar medium and synthetic medium for nocardia-like bacteria (g/l: urea – 1,5, Na_2HPO_4 - 4, KH_2PO_4 - 3, $MgSO_4$ –1, glucose – 30, saccharose – 10, $FeCl_3$ – 8 mg/l, B_1 – 1mg/l) were used to cultivate microorganisms.

To reveal growth ability of cultures at high osmotic pressure microorganisms were grown on agar media at different concentrations of NaCl (1M, 1.5M and 2M) in thermostat, at 28-30°C. The plates were inspected for growth after a week.

Growth intensity of cultures on solid nutritional media was estimated visually according to 4-point system.

To identify growth ability of cultures at different values of pH (2.0, 4.0, 6.0, 8.0, 10.0) of media, microorganisms were grown in 750-ml flasks, containing 50 ml of corresponding liquid media on circular rotator (180 revolutions per minute), at 28-30°C for 7 days. After sterilization of the media pH was adjusted by concentrated HCl and 1M NaCl.

Liquid media were inoculated with 10% of bacterial suspension at exponential growth phase. Inoculum was obtained on suitable media containing carbohydrates. Biomass in liquid media was determined by weighted method.

The ability of cultures to grow aerobically on a particular carbon compound supplied as a sole source of energy was tested on solid media.

The ability to grow on the various nitrogen compounds was tested on agar media. The basal medium was mineral medium with glucose, containing one of the sources of nitrogen.

The ability to grow on the various phosphorous compounds was tested on agar media with nitrogen base medium, containing one of the sources of phosphorus.

The incubated plates were inspected for growth at the different sources of carbon, nitrogen and phosphorus in a week's time. Growth intensity of cultures was estimated visually according to 4-point system.

Results and Discussion

Accumulation of biomass by microorganisms as a result of their relationship with substrates is basis for biodegradation of organic toxicants, since without of biomass synthesis it is impossible to synthesize enzymes participating in the process of biodegradation [V.Yarovenko et al., 1996].

To reveal the capability of cultures at different values of osmotic pressure the microorganisms were grown on agar media, at 1 M, 1.5 M and 2 M concentrations of NaCl. Out of 55 strains of investigated microorganisms 78% of them were able to grow on nutritional media containing 1 M NaCl, 69% - at 1.5 M NaCl and 64% - at 2 M NaCl. The microorganisms distinguished by high biodegradative ability are given in Table 1.

At 1M and 1.5 M concentrations of NaCl the majority of the microorganisms developed well. The obtained results showed that according to Kushner's classification [D.Kushner, 1981]. The most of presented microorganisms should be referred to moderate gallotolerants.

To reveal capability of growth in cultures possessing high-biodegradative ability the microorganisms were grown at different values of cultivation media pH 2.0, 4.0, 6.0, 7.0, 8.0 and 10.0. The results are given in Fig. 1.

Table 1. Influence of NaCl Concentration on Biomass Accumulation

NaCl (M)	Growth characteristics	Conditional N of the cultures
1M	Intensive growth	23, 124, 136, 140, 227, 235, 245
	Normal growth	13, 44
	No growth	-
1.5 M	Intensive growth	227, 245
	Normal growth	13, 23, 124, 136, 140, 235
	No growth	44
2 M	Intensive growth	227, 245
	Normal growth	13, 23, 124, 136, 140, 235
	No growth	44

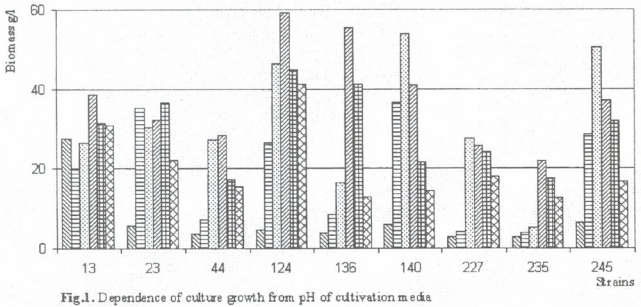

Fig.1. Dependence of culture growth from pH of cultivation media

pH 2,0 pH 4,0 pH 6,0 pH 7,0 pH 8,0 pH 10,0

At extreme values of pH the cultures N13 (27.5 g/l at pH 2 and 30.9 g/l at pH 10) and N 124 (41.3 g/l at pH 10) grew best of all. The former might be considered as acid tolerant and the latter alkaline tolerant. For the majority of investigated microorganisms the optimum pH is in the scope of neutral value (6.0-7.0). However, they might be developed without significant decrease of productivity varying at wide range of pH media, from 4.0 to 8.0.

It should be taken into consideration that the microorganisms tend to neutralize pH of cultivation media. This process proceeds more intensively at pH 4.0 and 8.0. It might be an explanation for almost all strains, which grew well at pH 4-8, forming the maximum of biomass. At different values of pH the change of colour of the culture suspension occurs.

The capability of microorganisms possessing high ability to utilise organic toxicants (mineral oil or TNT) and grow on different sources of carbon has been studied. Microorganisms were grown on corresponding mineral media in the presence of different carbohydrates, hydrocarbons, oil products, alcohols, organic acids and salts of organic acids as a sole source of carbon in the amount of 1%, except those for the negative controls, which have no carbon source added, and also for the positive controls, containing glucose. Culture growth was estimated visually

by 4-point system: - - no growth, 1 - poor growth, 2 - growth, 3 - intensive growth, 4 - heavy growth. Results are given in Table 2.

37 carbon compounds were used in order to characterize each strain. On the assumption of the data it might be concluded that the majority of microorganisms do not posses particular speciality and are characterised by the ability to develop well on the most of investigated sources of carbon. The strains grew well on sugars: glucose, fructose, saccharose and rhamnose; the worst growth was obtained on cellulose. Dulcite showed the worst utilization among alcohols; acetic acid, gallic acid and butyric acid were the worst among carbonic acids. The strain under conditional numbers 44 and 235 appeared the most exacting to sources of carbon.

It should be mentioned that all cultures capable for degradation of mineral oil are developed well on media containing saturated carbohydrates as a sole source of carbon, particularly C_{14}-C_{16} alkanes and oil products.

Among of nitrogen sources were tested 15 different organic and inorganic nitrogen-containing compounds as a sole source of nitrogen in the amount of 0.028% at the final concentration. The negative control was basal media without nitrogen sources (Table 3). The most of microorganisms are characterised by the ability to develop well on the majority of tested sources of nitrogen. The best was inorganic nitrogen as NO_3^- or NH_4^+ but not NO_2^-. The intensive growth was revealed on media containing amino acids: alanine, asparagine, aspartic acid, arginine, glycine and the worst growth – tyrosine, valine, cysteine and leucine.

K_2HPO_4 and KH_2PO_4 were tested as a source of phosphorus. It appeared that all microorganisms mentioned above grew well on the media containing one of these sources of phosphorus.

Thus, as it is shown from the results of our experiments the tested microorganisms don't display special traits. They are able to grow and degrade organic compounds (mineral oil or 2,4,6-trinitrotoluene or both of the toxicants) at wide range of temperature, pH of cultivation media, osmotic pressure and also to apply different sources of carbon, nitrogen and phosphorus for growth, which increase expectancy of their existence and development of biodegradative capability under natural conditions.

Table 2. The ability of bacteria to assimilate organic compounds as a sole source of carbon

Conditional № of cultures	Sources of carbon																						
	Pentose			Hexose			Disaccharides		Polysaccharides		Hydrocarbons							Oil products			Control
	L(1) arabinose	D(1) xylose	L(1) rhamnose	D(1) glucose	D(-) fructose	Saccharose	D(1) lactose	D(+) maltose	Galactose	Soluble starch	Cellulose	C_6	C_{11}	C_{13}	C_{14}	C_{15}	C_{16}	Mineral oil	Crude oil	Diesel fuel	
13	-	3	1	4	4	4	1	4	3	-	-	-	1	3	3	4	4	3	1	4	-
23	4	3	3	4	4	4	3	4	2	4	2	-	3	3	3	4	4	3	1	4	-
44	4	1	-	4	3	-	-	-	4	-	-	1	1	-	1	1	-	1	2	2	-
124	1	1	1	4	4	3	-	2	2	1	1	1	4	2	4	4	4	3	2	4	-
136	3	2	4	3	3	4	3	4	3	3	-	1	2	2	1	2	1	-	2	2	-
140	3	4	2	4	3	3	4	2	3	3	1	1	-	1	1	1	1	-	-	1	-
227	-	1	1	4	2	2	-	1	2	1	-	1	3	2	4	4	4	4	3	4	-
235	-	-	-	4	1	2	-	2	-	-	-	-	3	2	4	4	4	4	4	4	-
245	3	4	2	4	3	3	3	3	4	2	-	-	2	-	3	2	1	3	2	1	-

Table 2 (continue)

Conditional № of cultures	Sources of carbon																		Control
	Alcohols								Organic acids						Salts of organic acids				
	Ethanol	Butanol	Propanol	D(-) sorbite	D(-) mannitol	Dulcite	Inositol	Glycerol	Acetic	Stearic	Gallic	Capronic	Butyric	Palmitic	Citrate of Na	Malate of Na	Oxalate of Na		
13	2	2	2	4	4	2	4	3	1	2	1	3	2	2	4	2	2		-
23	3	3	2	4	4	-	3	3	1	2	3	2	3	2	4	2	2		-
44	3	2	1	4	4	-	4	3	3	2	1	1	-	1	3	2	1		-
124	4	4	3	3	3	1	2	1	-	1	2	3	1	3	2	3	2		-
136	2	3	2	3	3	1	2	-	-	2	1	1	1	1	3	2	1		-
140	2	3	2	1	3	1	2	1	2	2	-	2	1	2	1	2	1		-
227	2	2	1	1	1	1	-	1	1	2	1	1	1	1	-	1	1		-
235	-	1	1	-	1	-	-	1	2	-	1	1	-	1	-	1	1		-
245	3	4	2	-	3	3	2	1	-	2	2	2	1	3	-	2	1		-

Table 3. Capability of bacteria to assimilate organic and inorganic compounds as a sole source of nitrogen and phosphorus

Conditional № of cultures	Sources of nitrogen															Control
	DL- alanine	L-asparagine	L- aspartic acid	DL-leucine	D- tyrosine	DL- valine	L- arginine	glycine	L- cysteine	Methionine	DL-lysine	$NaNO_3$	$NaNO_2$	NH_4Cl	$(NH_4)_2SO_4$	
13	2	4	2	2	3	3	2	3	2	3	3	4	-	4	1	1
23	3	4	3	4	3	3	4	4	3	3	4	4	2	4	4	1
44	4	1	3	-	-	-	4	3	1	-	1	4	-	4	4	-
124	4	4	4	4	3	3	4	3	4	4	4	4	3	4	4	-
136	4	4	4	-	-	-	4	3	1	3	-	4	3	4	4	-
140	4	2	4	2	2	2	3	4	2	2	1	4	4	2	3	1
227	4	2	3	2	2	1	4	4	1	2	2	4	1	4	4	-
235	2	2	3	2	2	1	4	4	1	2	2	4	-	4	4	-
245	4	4	1	-	2	2	4	3	1	2	1	2	1	4	4	-

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ზოგიერთი ფიზიკო-ქიმიური ფაქტორის გავლენა 2,4,6-ტრინიტროტოლუოლისა და მინერალური ზეთის დეგრადაციის უნარის მქონე მიკროორგანიზმების განვითარებაზე

ვარსიმაშვილი ხ., თინიკაშვილი ლ., ამირანაშვილი ლ., გაგელიძე ნ., კირთაძე ე., ხატისაშვილი გ., ღოღობერიძე მ.

საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 03.05.2004)

რეზიუმე

შესწავლილ იქნა ზოგიერთი ფიზიკურ-ქიმიური ფაქტორის (საკულტივაციო არის pH, ოსმოსური წნევა, ნახშირბადის, აზოტისა და ფოსფორის წყაროები) გავლენა იმ მიკროორგანიზმების კულტივირებისა და განვითარების პირობებზე, რომლებიც 2,4,6-ტრინიტროტოლუოლისა (ტნტ) და მინერალური ზეთის დეგრადირების მაღალი უნარით გამოირჩევიან. დადგენილია, რომ გამოკვლეულ მიკროორგანიზმებს არ ახასიათებთ ვიწრო სპეციალიზაცია; მათ გააჩნიათ აღნიშნული ორგანული ნივთიერებების შემცველ არეებზე ზრდისა და აგრეთვე ამ ნივთიერებების დეგრადაციის უნარი pH-ის და ოსმოსური წნევის ფართო დიაპაზონში. აღნიშნულ მიკროორგანიზმებს შეუძლიათ ზრდისათვის გამოიყენონ ნახშირბადის, აზოტისა და ფოსფორის მრავალი წყარო, რაც ბუნებრივ პირობებში ზრდის მათი არსებობისა და ბიოდეგრადაციული უნარის გამოვლენის ალბათობას.

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Zoology

THE ECOLOGICAL STUDY OF ASIA MINOR FOREST MOUSE (*APODEMUS MISTACINUS DANF. ET ALST*) OF THE SATAPLIA RESERVE

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(Received May 10, 2004)

Abstract

The bioecological study of Asia Minor forest mouse *(Apodemus mistacinus Danf.et Alst)* was conducted in 1996-1999. 7 habitats were chosen: hornbeam forest with admixture of other tree species, oriental hornbeam forest, beech forest with blackberry cover, alder forest with blackberry cover, pine forest, beech forest with box-tree understory, maize field of he village Khomuli. Asia Minor forest mouse usually lives in the beech forest with box-tree understory and pine forest with fern cover. In the autumn they migrate to the maize fields; maximal reproduction takes place from July till August-September; Autumn ecdysis goes in November. They use two kinds of nests – hot-nest and shelter nest. They are fed on plant, mainly on the seeds and in the spring – greenery, that is the reason of negative influence of regeneration-restoration of the forest.

Key Words: *Apodemus mistacinus Danf. et Alst.*, bioecology, habitat, ecdysis.

Introduction

The bioecological study of Asia minor forest mouse was poor, because of their rare spread in Georgia [Kurashvili et al., 1981, Burduli, 2000] *Apodemus mistacinus Danf. et Alst* is extended in Balkans, Asia Minor, West Caucasus; in Georgia they were found in Ajara, Colchi foothill, on the mountain-ridge of Meskheti, Surami, Lechkhumi and Racha, the mouth of the rivers Khobi and Tskhenistskali [Abuladze, 2001, Chkheidze, 1997]. The number of Asia Minor forest mouse is not high. They are twice bigger than home-mouse (*Mus. musculus*), their back is gray, belly – white, sometimes with yellow shade. They mainly live in forest biotopes, deciduous and mixed forests, rocky and stony places. They are active in the evening and night.

The food remains near the nests and content of stomach of forest mouse was studied. The number of male and female sexually mature individuals were compared.

Materials and Methods

The bioecological study of Asia Minor forest mouse was conducted in 1996-1999, from May till the end of November in the reserve of Sataplia. 6 somehow different habitats were chosen: hornbeam forest with admixture of other tree species (chestnut tree, beech tree, box-tree), oriental

hornbeam forest, beech forest with blackberry cover, alder forest with blackberry cover, pine forest with fern, beech forest with box-tree understory and maize field of the village Khomuli. They were caught with special traps. The traps were put 5 times a month in the evening and were registered from 8 till 10 a.m. During experiment in every habitat 10 traps were put every night. Registration was carried out on the base of 50 trap-night.

Results and discussion

During our observation 295 specimen were caught and among them 20 were resected. Distribution of Asia minor forest mouse in different habitats by month is given in table 1.

As it is seen from the tables forest mice are spread in the beech forest with box-tree understory and pine forest with fern cover. Their amount reaches maximum in August. From August they migrate to the to the adjacent regions of the reserve to the maize fields and in December return to the reserve. The reason of Asia Minor forest mouse spreading in the beech forest with the box-tree understory is the amount of food and shelter. The same habitat is the beech forest with blackberry cover, which is occupied by another species of forest mouse *Apodemus silaxicus L.*

Among specimen catch in November there were the ones which have Autumn ecdysis. The ecdysis period is very short and these specimen are found only during four days.

Table 1. Distribution of Asia Minor forest mice *(Apodemus mistacinus Danf. et Alst)* in different habitat by month; observations were carried out in 1996, 1997, 1998, 1999

1996

№	habitat	Trap-night / catch individuals							total
		month							
		V	VI	VII	VIII	IX	X	XI	
1	hornbeam forest with admixture of other tree species	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
2	oriental hornbeam forest	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
3	beech forest with blackbarry cover	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
4	alder forest with blackberry cover	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
5	pine forest	50/0	50/0	50/1	50/3	50/0	50/0	50/0	350/4
6	pine forest with box-tree	50/7	50/9	50/7	50/12	50/8	50/6	50/7	350/56
7	maize fields of the village Khomuli	50/0	50/0	50/0	50/1	50/3	50/6	50/4	350/14
Total		7	9	8	16	11	12	11	74

1997

№	habitat	Trap-night / catch individuals							
		month							total
		V	VI	VII	VIII	IX	X	XI	
1	hornbeam forest with admixture of other tree species	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
2	oriental hornbeam forest	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
3	beech forest with blackbarry cover	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
4	alder forest with blackberry cover	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
5	pine forest	50 / 0	50 / 0	50 / 1	50 / 3	50 / 0	50 / 0	50 / 0	350 / 5
6	pine forest with box-tree	50 / 3	50 / 7	50 / 9	50 / 7	50 / 8	50 / 9	50 / 7	350 / 57
7	maize fields of the village Khomuli	50 / 0	50 / 0	50 / 0	50 / 4	50 / 3	50 / 6	50 / 2	350 / 15
Total		350 / 3	350 / 8	350 / 10	350 / 14	350 / 12	350 / 14	350 / 11	350 / 72

1998

№	habitat	Trap-night / catch individuals							
		month							total
		V	VI	VII	VIII	IX	X	XI	
1	hornbeam forest with admixture of other tree species	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
2	oriental hornbeam forest	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
3	beech forest with blackbarry cover	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	50 / 0	350 / 0
4	alder forest with blackberry cover	50 / 1	50 / 1	50 / 3	50 / 2	50 / 1	50 / 3	50 / 1	350 / 12
5	pine forest	50 / 7	50 / 10	50 / 11	50 / 12	50 / 8	50 / 5	50 / 8	350 / 61
6	pine forest with box-tree	50 / 7	50 / 9	50 / 7	50 / 12	50 / 8	50 / 6	50 / 7	350 / 56
7	maize fields of the village Khomuli	50 / 0	50 / 0	50 / 0	50 / 3	50 / 4	50 / 2	50 / 0	350 / 9
Total		350 / 8	350 / 11	350 / 14	350 / 17	350 / 13	350 / 10	350 / 9	350 / 82

N	habitat	Trap-night / catch individuals — month V	VI	VII	VIII	IX	X	XI	total
1	hornbeam forest with admixture of other tree species	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
2	oriental hornbeam forest	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
3	beech forest with blackbarry cover	50/0	50/0	50/0	50/0	50/0	50/0	50/0	350/0
4	alder forest with blackberry cover	50/1	50/1	50/3	50/2	50/1	50/3	50/1	350/12
5	pine forest	50/1	50/2	50/2	50/1	50/2	50/3	50/2	350/13
6	pine forest with box-tree	50/5	50/6	50/7	50/8	50/5	50/7	50/6	350/44
7	maize fields of the village Khomuli	50/0	50/0	50/0	50/1	50/4	50/2	50/3	350/10
Total		350/6	350/8	350/9	350/10	350/11	350/12	350/11	350/67

As our observations were carried out from May, the spring ecdysis wasn't noticed (usually spring ecdysis among mouse-like rodents occurs at the end of March and the beginning of April). From received material 161 were female and 134 – male (table 2).

Table 2. The number of catch individuals in different years by month and sex

	Catch individuals														
	1996	♀	♂	1997	♀	♂	1998	♀	♂	1999	♀	♂	total catch	♀	♂
May	7	5	2	3	2	1	8	5	3	6	4	2	24	16	8
June	9	5	4	8	5	3	11	6	5	8	3	5	36	19	17
July	8	3	5	10	3	7	14	7	7	9	5	4	41	18	23
August	16	10	6	14	8	6	17	10	7	9	5	5	57	39	24
September	11	5	6	12	5	7	13	8	5	11	6	5	47	24	23
October	12	7	5	14	8	6	10	6	4	12	6	6	48	27	21
November	11	6	5	11	7	4	9	5	4	11	6	5	42	24	18
Total	74	41	33	72	38	34	82	47	35	67	35	32	295	161	134

251 specimen were mature and 44 young. Probability of the catch of young mice in the trap is low.

The nests of Asia Minor forest mouse were studied. They usually make their nests under stones, the roots of blackberry bush, rotten tree. The nests are of two types – hot-nests and for food

stock. For the building of hot-nest the forest mouse uses the area between stones which is covered with dry leaves of the beech, it has several exits. In the nest both individuals – males and females live (at the exit of one hole we have caught both individuals). Another type of nest, different from the hot-nest and used for food stock and as a shelter, has a complicated labyrinth of pathways with widenings for food storing in some places and several exits. Sometimes this net is connected with hot-net by 5-10m pathway.

The resection of Asia Minor forest mouse and analysis of stomach content showed that they are fed only on plant, mainly with the seeds of plant, but in the spring they eat greenery. Resection of 20 individuals has shown that forest mouse chooses their meal depending on the season of the year. The data are presented in the table 3.

Table 3. The data of resection by seasons

seasons	Number of resected mouse	animal food	plant food	
			greenery	seeds
Spring	5	—	5	5
Summer	5	—	—	5
Autumn	5	—	—	5
Winter	5	—	—	5

Thus, we can conclude, that Asia Minor forest mice live in the beech forest with box-tree understory and in the pine forest with fern cover of the Sataplia reserve; in the Autumn they migrate to the maize fields of the village Khomuli. The reproduction reaches maximum in August-September; autumn ecdysis occurs in November. They use two kinds of nests: hot-nest and the nest for food stock. They feed on plant, mainly the seeds and in the spring they eat greeny, which is the reason of their negative influence on regeneration-restoration and the harvest of the maize.

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სათაფლიას სახელმწიფო ნაკრძალში მცირეაზიური ტყის თაგვის (*Apodemus mistacinus Danf et Alst*) ეკოლოგიური გამოკვლევა

გორდაძე ე., ჟორჟოლიანი ც.

ბიოლოგიის კათედრა, ქუთაისის აკაკი წერეთლის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

ჩატარებულია მცირეაზიური ტყის თაგვის ბიოეკოლოგიური შესწავლა სათაფლიის ნაკრძალში 1996-1999 წლებში. შერჩეულია მცენარეთა მეტნაკლებად განსხვავებული შეიდი სტაცია: რცხილნარი შერეული ჯიშებით, ჯაგრცხილნარი, წიფლნარი მაყვლის საფარით, მურყნარი, ფიჭვნარი გვიმრის საფარით, წიფლნარი–ბზის ქვეტყით და სოფელ ხომულის სიმინდის ყანა. მცირეაზიური ტყის თაგვი ძირითადად ცხოვრობს წიფლნარში ბზის ქვეტყით და ფიჭვნარში გვიმრის საფარით. შემოდგომაზე ახდენს მიგრაციას ხომულის სიმინდის ყანებში. მათი გამრავლება მაქსიმუმს აღწევს ივლისიდან აგვისტო–სექტემბერში. საშემოდგომო განაური ახასიათებთ ნოემბრის თვეში. აგებენ ორი სახის ბუდეს - სათბურს და თავშესაფარს. იკვებებიან მცენარეთა თესლებით, გაზაფხულზე მწვანე მცენარეებით, რის გამოც უარყოფით გავლენას ახდენენ ტყის აღდგენა–განახლებაზე.

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Zoology

STUDY OF ARTIFICIAL AND NATURAL REPRODUCTION OF STURGEON FISHES IN THE RIVER RIONI

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(Received May 3, 2004)

Abstract

The reasons of the decrease of stocking of Sturgeon fishes in the South-East of the Black Sea and the river Rioni has been discussed. Grown by artificial reproduction 117000 viable Colkhi Sturgeon larvae have been set free. Natural and artificial reproduction of Sturgeon fishes in the r. Rioni have been studied. Such quantity of artificial reproduction of the Colchi Sturgeon has never been got before.

Key words: Sturgeon fishes, artificial fertilization, *Acipencer güldenstädti colchicus V.Marti*

Introduction

There are five species of sturgeon fishes and two subspecies found in the Black Sea: Atlantic Sturgeon (*Acipencer sturio Linne*), 2 subspecies of Russian Sturgeon – Colchi and Persian (*Acipencer güldenstädti colchicus V.Marti*), *Acipenser nudiventris Lovetzky, Acipencer stellatus Pallas* and *Huso huso Linne.* The unique and endemic species of the population of sturgeon - *Acipencer sturio linne, Acipencer nudiventris* – reached the critical level. The rest of the species exist as relicts and they are in danger to be vanished.

In 60-ies in the Black Sea coast were caught approximately 600 tone of statistically registered cartilaginous fishes: sturgeons, salmons, etc. Today fishing of these species is poaching and only four species of fishes of the Black sea is profitable. It was shown by the various researchers that the main reason of the fish number and the fishery area decrease is hard fishing.

In 60-ies a number of arrangements were conducted with the purpose of conservation of the acipenserid fishes in the south-east sector of the Black Sea. In 80-ies was substantiated the evidence of construction of a sturgeon-breeding farm on the r.Rioni. Five-mile coastal zone from Poti-Anaklia to Ochamchire, as well as from the mouth of the river Rioni to the village Vartsikhe was destined as protected area (natural reserve). But unfortunately from 1 December till 1 April the fishing (including illegal fishing) on khamsa was allowed, that causes danger of vanishing Sturgeon and Salmon fishes

Materials and Methods

To carry out biotechnological process of artificial reproduction of Sturgeon fishes Ginsburg and Detlaf method was used [Ginsburg, Detlaf, 1969]. For conservation of sire of Colchi Sturgeon fishes the method with "КаспНИРХ" modification was used [Ninua et. al., 2000].

Results and Discussion

On the base of analysis of existing conditions the program "Conducting restocking process by artificial reproduction of acipenserid fishes of Georgian Black Sea-coast sector and studying conditions of natural spawning" was worked out.

Due to different economic and political reasons the fish-breeding farm was not functioning. In 1998 recreation of technological process was conducted. This work was supported by the Environmental Protection Institutions of Germany and Georgia.

8 sexually mature individuals (sire) of Sturgeon were caught at the mouth of the r.Rioni:

1. *Acipenser sturio Linne* – 142cm. ♂
2. *Acipenser stellatus pallas* – 115cm. ♀
3. *Acipencer güldenstädti colkhicus V.Marti* (5 individuals)–106, 133, 134, 135, 144cm. ♂
4. *Acipencer güldenstädti colkhicus V.Marti* - 160cm. ♀

It is worth mentioning that among sexually mature individuals (sire) one male of *Acipenser sturio Linne* and one female of *Acipenser stellatus pallas* were caught. The experiment was carried on *Acipencer güldenstädti colchicus V.Marti* . During the period of initiation and morphometric analysis the fishes were put in plastic basin of sizes 2x2x1m with artesian well water and at 14-15^{0}C. For female initiation suspension containing 50 mg of powdered hypophysis diluted in 2,5ml. distilled water was used; for one male (106 cm) 25mg hypophysis was diluted in 1ml distiled water and for another male (135 cm) - 30mg hypophysis in 1,5 ml. water. During the hypophysis solutiom treatment female and two male individuals of *Acipencer güldenstädti colkhicus V.Marti* were initiated in the back muscle between the 4th and 5th scale. Observing the fish behavior, maturity was controlled visually. The maturity time was determined according to the Ginsberg - Detlaf diagram, where maturity time is considered to be 36 hours. The female maturity time was 34h and male – 24h. To rescue female, Caesarian section was made using method with "КаспНИРХ" modification. Nine cross stitches were made on the section with triple knot on the last stitch. After operation the fishes were put in the basin under maximal flow of water. After 11-day observation, insuring in their viability, on 12th day they were let in the river. 4,5g of matured spawn was fertilized by sperm (from one male 110ml sperm was received and from another – 130ml). The sperm activity determined by Persov scale was 5 units.

For fertilization the sperm of both males was mixed in equal quantities. To 1kg spawn 10ml of sperm water solution (in ratio 1:150) was added. Fertilization was lasted 5min. Residual sperm and the silt for ungluing diluted in water (accounting 1kg spawn per 1l) was added to the suspension. The ungluing lasted 40min, adding 1-1,5l pure water every 15min. After this procedure pure, unglued spawn was placed in special boxes. The analysis showed that fertilization was 92%. The sterility of artesian water determined the normal incubation, inhibition of "Saprelenia" and high fertilization (92%).

The observations were carried out on every 36 stages of fertilization. On gastrulation stage fertilization was 86%, on heart-pulsation stage – 90%. On 7th day after fertilization hatching of larvae began and ended in 12 days. Embryonic development lasted 160 hours. One-day larvae weighted 35mg were put in radial basins. After sucking of yolk valve (on 3-4 day) they were feed with juveniles Dafnia. On the 7th day larvae were put in the reservoir with Dafnia culture, mineral

fertilizer (50kg/ha amonium saltpetre and 150-200kg/ha superphosphate) and left there for 7 weeks. They were fed with dafnia and invertebrates grown on fertilizers. More than 117000 larvae with size 12-16 cm and weight 5-6,5 g were let in natural conditions in September. This is the highest index for artificially grown Sturgeon fishes.

The biological analysis was carried on larvae and juveniles caught at the mouth of the r. Rioni. To determine the degree of Sturgeon fishes reproduction, their viability and the rate of their rolling down in to the sea was studied.

The natural process of coming down from the river to the sea of Sturgeon fishes lasted from a few days to a few months depending on species. The control catch was carried out from May till October.

Our observations have shown that natural reproduction of Sturgeon fishes in the r. Rioni still occurs. It was conformed that in our experiments artificially grown larvae is more viable and growth rate is higher than the natural ones (Table 1 and 2). Thus, the index of returning from the sea to the river for artificially grown fishes would be much higher.

For the effective reproduction of Sturgeon fishes it is necessary to build a research base where it will be possible to receive spawn and fertilize in the extreme conditions.

Table 1. Quantitative analysis of natural reproduction of Sturgeon fishes distributed in the r. Rioni

months	larvae		
	size (cm)	weight (g)	amount
May	0,08-0,9	0,1	10
June	1,0-2,0	1,9	5
July	1,0-3,0	2,5	30
August	2,0-3,5	3,8	30
September	3,5-4,0	4,9	15

Table 2. Produced by artificial reproduction Larvae of *Acipencer güldenstädti colkhicus V.Marti* let in the r. Rioni

months	size (cm)	weight (g)	amount
September (coming down from the river to the sea)	5,0-6,5	10-16	117000
October (control catch)*	3,0-6,8 5,0-7,2	4,4-7,5 14-18	80 86

*The control catch was carried out twice; in the control catch part of natural reproductive larvae which were gone late in the sea is presented.

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მდინარე რიონში ზუთხისნაირთა ხელოვნური და ბუნებრივი აღწარმოების შესწავლა

ნინუა ნ., გოგინაშვილი თ., ბოჭორიშვილი ვ.

ზოოლოგიის განყოფილება, საქართველოს სახელმწიფო მუზეუმი

(მიღებულია 03.05.2004)

რეზიუმე

განხილულია შავი ზღვის სამხრეთ-აღმოსავლეთ ნაწილისა და მდ. რიონში გავრცელებულ ზუთხისებრთა 5 სახეობის მარაგის შემცირების მიზეზები. ხელოვნური აღწარმოების გზით გამოზრდილი და ბუნებაში გაშვებულია 117000 სიცოცხლისუნარიანი კოლხური ზუთხის ლიფსიტა. შესწავლილია ზუთხისნაირთა ხელოვნური და ბუნებრივი აღწარმოება მდინარე რიონში. რიონის ზუთხსაშენ ქარხანაში კოლხური ზუთხის ხელოვნური აღწარმოება ასეთი რაოდენობით პირველადაა ჩატარებული.

ინსტრუქცია ავტორთათვის

სამეცნიერო ნაშრომს ჟურნალი ბეჭდავს ინგლისურ ენაზე, მას უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე, სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება, საკვანძო სიტყვათა მოკლე (4-6) სია.

სამეცნიერო წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (introduction), მასალა და მეთოდები (materials and methods), შედეგები და მათი განხილვა (results and discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით. ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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ჟურნალის ბეჭდვა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს ნაშრომზე დადებითი რეცენზიის მიღებისთანავე. ნაშრომის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში ნაშრომი, დამატებით გაეგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, ნაშრომი არ გამოქვეყნდება.

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