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Biochemistry

DYNAMICS OF ACCUMULATION OF BLACK TEA PIGMENTS DURING TEA (*CAMELLIA SINENSIS L.*) LEAF PROCESSING

MCHEDLISHVILI N.[1], OMIADZE N.[1], GULUA L.[1], NISHNIANIDZE N.[1], REKHVIASHVILI M.[2], PRUIDZE N.[1]

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(Received June 14, 2004)

Abstract

Accumulation of the black tea pigments theaflavins and thearubigins during fermentation of fresh and coarse tea leaves was investigated. Theaflavins in higher concentrations are accumulated in fresh tea leaves than in coarse leaves. Maximal quantity of theaflavins (0.91%) is detected in fresh tea leaves after an hour of fermentation. The content of thearubigins in fermented fresh tea leaves varied from 11% to 16% and in the coarse leaves from 13% to 17%.Theaflavins and thearubigins were isolated from the black instant tea powder. These compounds are found to be effective inhibitors of tea leaf phenol oxidase and peroxidase.

Key words: theaflavins, thearubigins, tea, phenol oxidase, peroxidase.

Introduction.

During the rolling-fermentation stage of black tea manufacture the cellular integrity of the leaves is disrupted by interaction of flavan-3-ol derivatives, catechins stored in the vacuoles with polyphenol oxidase (E.C. 1.14.18.1. monophenol dihydroxyphenilalanine: oxygen oxydoreductase) and peroxidase (EC 1.11.1.7) released from the chloroplasts. In the presence of atmospheric oxygen polyphenol oxidase catalyze the oxidation of tea catechins, especially (–) epigallocatechin gallate, into o-quinones, which in turn are polymerized into theaflavins [Subramanian, at al. 1999; Keegel, 1983]. Theaflavins are catechin dimmers that contribute to the yellow-orange color of black tea liquors. However, theaflavins are not stable and can be further oxidized to thearubigins, which are a heterogeneous group of catechin polymers of unknown structure, which can be also formed directly from catechins [Goodsall, *et al.* 2000; Goodsall and Safford, 1998]. Thearubigins are pigments that contribute to the brown color of black tea liquors.

Both, theaflavins and thearubigins, appear to be the major pigments of black tea since aroma, liquor, and taste of the product depend on theaflavins and thearubigins content.

The aim of this work is to study dynamics of accumulation of the theaflavins and thearubigins in the leaves during tea fermentation, to isolate these pigments from black tea and to establish their effect on the activity of the enzymes polyphenol oxidase and peroxidase.

Materials and methods.

Fresh and coarse tea (*Camellia sinensis*) leaves of different vegetation period were used in the experiments. The tea leaves were collected in West Georgia in September-October period. Withering and fermentation processes were conducted according to the common technology [Keegel, 1983].

Theaflavins and thearubigins were isolated from the black instant tea powder. 20 g black tea powder was resuspended in 400 ml water. The aqueous fraction was then extracted successively with 2x400 ml chloroform and 4x400 ml ethyl acetate. The chloroform removes caffeine and any residual lipid. The ethyl acetate fractions containing theaflavins and thearubigins were combined and re-extracted with an equal volume of water, then dried over approximately 2 g anhydrous $MgSO_4$, resuspended in water, freeze dried and used as thearubigins preparation. To isolate the theaflavins the thearubigins preparation was solved in water and loaded on the silicagel column. Elution of the theaflavins from the column was performed with methanol.

Theaflavins and thearubigins quantitatively were determined spectrophotometrically. Phenol oxidase and peroxidase crude preparation from tea leaves was obtained as described by [Pruidze, 1987]. The polyphenol oxidase and peroxidase activities were determined spectrophotometrically [Lanzarini, *et al.*, 1972; Evans and Aldridge, 1965]. Protein content was determined by Amino Black reagent.

Received data were treated statistically. Presented data are the mean of three replicates ± standard deviation. All calculations were performed with Microsoft Excel (Version 4, statistical functions, Microsoft Corp., Redmond, WA, USA).

Results and discussion.

Study of dynamics of accumulation of the black tea pigments, theaflavins and thearubigins, during fermentation of fresh and coarse tea leaves showed that theaflavins were accumulated in higher concentrations in fresh tea leaves (0.17-0.91%) than in coarse leaves(0.09-0.22%). Maximum quantity of theaflavins (0.91%) were detacted in fresh leaves after an hour of fermentation. Maximum amount of theaflavins in coarse leaves was found after 1.5 hour of fermentation and it did not exceed 0.22% of dry matter of the leaves. The content of thearubigins in fermented fresh tea leaves varied from 11% to 16% and in the coarse leaves from 13% to 17% (Tab.1).

Table1. Dynamics of accumulation of theaflavins and thearubigins in fresh and coarse tea leaves during fermentation.

Duration of fermentation, min	Fresh tea leaves		Coarse tea leaves	
	Theaflavins, % of dry matter	Thearubigins, % of dry matter	Theaflavins, % of dry matter	Thearubigins, % of dry matter
20	0.17±0.02	11±0.2	0.09±0.01	17±0.4
40	0.65±0.06	16±0.3	0.10±0.01	14±0.3
60	0.91±0.07	16±0.3	0.12±0.01	16±0.3
90	0.49±0.05	11±0.2	0.21±0.01	17±0.3
120	0.53±0.05	13±0.3	0.22±0.01	15±0.3
150	0.56±0.05	12±0.2	0.18±0.01	16±0.4
180	0.69±0.06	15±0.2	0.19±0.01	13±0.2

Effect of theaflavins and thearubigins isolated from black instant tea powder on the activity of tea leaf phenol oxidase and peroxidase was studied. According to the data (Fig.1)

theaflavins were more effective inhibitors of phenol oxidase than thearubigins. 50% inhibition of phenol oxidase activity was achieved at 0.35 mg/ml and 0.2 mg/ml concentrations of thearubigins and theaflavins respectively.

Peroxidase of tea leaf is inhibited by thearubigins and theaflavins with the same extent. 50% inhibition of peroxidase is observed at 0.01 mg/ml concentration of both thearubigins and theaflavins (Fig.2).

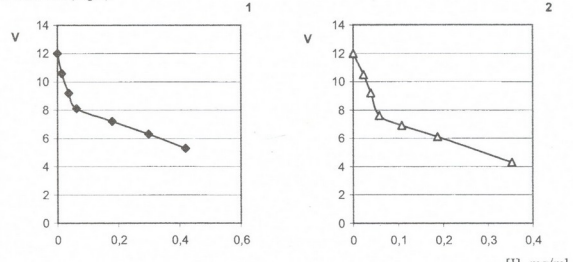

Fig.1. Effect of thearubigins (1) and theaflavins (2) on tea leaf phenol oxidase activity. V-specific activity, ΔE/mg protein/min

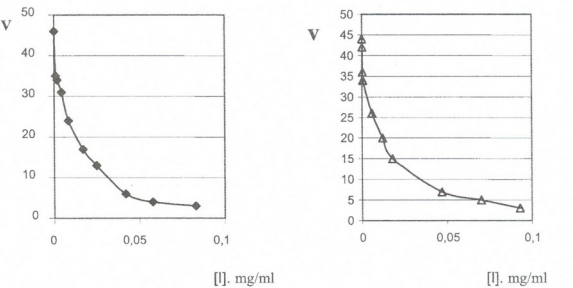

Fig.2. Effect of thearubigins (1) and theaflavins (2) on tea leaf peroxidase activity. V-specific activity, ΔE/mg protein/min

Thus, theaflavins and thearubigins, black tea pigments, accumulating in the fermented tea leaves during fermentation stage of black tea manufacture, cause inhibition of activity of the main redox enzymes of tea leaf, peroxidase and phenol oxidase, and reduce the fermentation rate.

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შავი ჩაის პიგმენტების დაბროვების დინამიკა ფოთლის გადამუშავების პროცესში

მჭედლიშვილი ნ.,[1] ომიაძე ნ.,[1] გულუა ლ.,[1] ნიშნიანიძე ნ.,[1]
რეხვიაშვილი მ.,[2] ფრუიძე ნ.[1]

[1]საქართველოს მეცნიერებათა აკადემიის ს. დურმიშიძის სახელობის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი,
[2]კვების მრეწველობის ინსტიტუტი

(მიღებულია 14.06.2004)

შესწავლილია ნაზი და უხეში ჩაის ფოთლების ფერმენტაციის პროცესში შავი ჩაის პიგმენტების დაგროვების დინამიკა. თეაფლავინები გროვდება უფრო დიდი რაოდენობით ნაზ ფოთლებში და მათი მაქსიმალური რაოდენობა (0.91%) შეინიშნება ფერმენტაციის დაწყებიდან ერთი საათის შემდეგ. თეარუბიგინების შემცველობა ფერმენტირებულ ნაზ ფოთლებში ცვალებადობს 11%-დან 16%-მდე, ხოლო უხეშ ფოთლებში - 13%-დან 17% -მდე. თეაფლავინები და თეარუბიგინები გამოყოფილია შავი ჩაის ფხვნილიდან. ნაჩვენებია, რომ ეს ნაერთები ჩაის ფოთლის ფენოლოქსიდაზასა და პეროქსიდაზას ეფექტური ინჰიბიტორებია.

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Biochemistry

KINETICS OF ENDOGENOUS FLAVONOID COMPOUNDS METABOLISM IN LEAVES OF GEORGIAN LEMON (*CITRUS LIMON BURM.*)

TARGAMADZE I., MZHAVANADZE V., ZAMBAKHIDZE N., TSIKLAURI G., PAPUNIDZE S., SHALASHVILI A.

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(Received June 10, 2004)

Abstract

The metabolism of main flavonoid glycosides - isoroifoline (apigenine-7-O-rutinoside), scolimoside (luteoline-7-O-rutinoside) and rutin (quercetine-3-O-rutinoside) was studied in Georgian lemon (*Citrus Limon Burm.*) leaves by labeled carbon atom. $^{14}CO_2$ was introduced into the cut shoots of lemon by a photosynthesis. Due to quantitative changes of isoroifoline, scolimoside, rutin and their total and specific radioactivity it was shown that these compounds undergo intensive metabolic changes in the leaves of lemon. The half-life of these substances were 30, 55 and 48 hours, respectively.

Key words: *flavonoids, metabolism, leaves, Citrus Limon Burm.*

Introduction

It is well known that flavonoid compounds are synthetised by higher plants and undergo intensive and various metabolic transformations, till cleavage of aromatic rings, using the products of cleavage as carbon and energy sources [Durmishidze S., 1984; Zapramet0v M., 1996;1993]. In lemon plants there is a significant amount of flavonoids and they are mainly flavanones, flavones and flavonols. Despite a great success reached in study of chemical nature of flavonoid compounds in citric plants, many questions of endogenous flavonoids metabolism in different parts of lemon still remain obscure. We established that the dominant compounds of Georgian lemon leaves flavonoids are glycosides: isoroifolin (apigenine-7-O-rutinoside), scolimoside (luteolin-7-O-rutinoside) and rutin (quercetine-3-O-rutinoside). The goal of the present work was to study metabolic transformations of isoroifolin, scolimoside and rutin.

Materials and methods

The techniques of "pulse labeling" was used, which allows to judge about the metabolism of flavonoid compounds on the grounds of changes in total and specific radioactivities. Radioactivity was introduced into the studied compounds by plant exposure in $^{14}CO_2$ atmosphere, generated from $Ba^{14}CO_3$ (10μCi). Cut seedlings of Georgian lemon were placed into glasses with water and exposed for 3 hr in a organic glass chamber (vol. 100 l) to diffuse sun light. After

stopping of exposure the air was pumped out and unabsorbed $^{14}CO_2$ was caught by alkali. Straight away after opening of a chamber the first sample was taken. The rest glasses with seedlings were kept in conditions of natural photoperiod during next 169 hours. During the test the leaves were in condition of normal turgor and did not differ from the freshly collected ones by their outward appearance. At definite time of intervals the samples of plant material (leaves) were selected, they were fixed by water vapour during 10-15 min and then dried in dark at room temperature. Weights (5g) of air-dried cut leaves in small packages, made up of filter paper were placed into Soxhlet apparatus extractor and exhaustively extracted by chloroform (35 hr). After extraction the packages with weights were dried in a hood and then their content was carried into flat-bottomed flasks (250 ml) and extracted 6 times with 80% methanol (60ml) on boiling water bath. The duration of each extraction was 30 minutes. Then the extracts were united, filtrated and steamed in vacuum at 40° till a small volume, and reduced by methanol to 20 ml. 60-100 μl of these solutions were put on chromatographic paper FN1 sheets and cromatographed in systems: n-butanol-acetic acid-water, 4:1:5 (I direction) and 15% acetic acid (II direction). The spots of the studied flavonoids were found in UV, they were cut out and eluted twice with methanol (10 ml) on boiling water bath. The duration of each extraction was 30 minutes. United extracts were steamed, the sediments were dissolved in 1 ml of methanol each and again chromatographed on paper FN1, using 30% acetic acid as a solvent for isoroifolin and rutin, but a system: ethylacetate - 85% formic acid - water, 10:2:3 – for scolimoside. The chromatogrammes were developed by 10% $AlCl_3$ in methanol. Isoroifolin, scolimoside and rutin spots were found in UV, they were cut out and placed into flasks (20 ml), 5 ml methanol and 10 drops of 10% $AlCl_3$ in methanol was added to each of them. Elution of flavonoids from paper was carried out at 2 hr infusion and further heating with back cooling on boiling water bath. Eluates were reduced to 5 ml by methanol and optical density of isoroifolin, scolimoside and rutin solutions was measured at 382, 420 and 430 nm, respectively, on spectrophotometer SF-26, in cuvettes with 1 sm absorption layer [Markham K.R., 1982]. Flavonoids content was defined by calibration graphics, built for pure samples of isoroifolin, scolimoside and rutin [Gamtsemlidze E., 2002].

Radioactivity was determined in scintillatory counter 1215 Rackbeta II (LKB Wallac), with 97% efficiency. The studied flavonoid glycosides, isolated and purified by two- and one-dimensional chromatography on paper were dissolved in 1 ml methanol. The solution was completely put into a vessel containing 10 ml of toluene scintillatory mixture for radioactivity counting. 4g of 2,5-diphenyloxalate and 100 ml of 1,4-bis-2(5-phenyloxazolil) of benzene was taken for 1l of toluene. Recurrence of each determination is 5-7 fold. Average comparative error of radioactivity measurement for isoroifolin, scolimoside and rutin is ±3,5%, ±4,3% and ±4,7% respectively.

Results and discussion

From date, presented on Fig1, it is evident that after exposure in $^{14}CO_2$ atmosphere (3hr) isoroifolin, scolimoside and rutin content is 0,29, 0,23 and 0,55 mg/g of leaves dry weight, respectively. At the same time rutin content is two times higher than isoroifolin and scolimoside ones. Further at exposure without $^{14}CO_2$ the content of the studied compounds increases first 23 hr, and then, almost till the end of the test is slightly changes. During the intervals from 25 to 121 hr the curves are almost parallel to absciss axis that may certify a dynamic balance between simultaneously carried out processes of biosynthesis and transformation of flavonoid compounds. After 121 hr exposure without $^{14}CO_2$ the content of the studied compounds decreases. Thus, during the experiment on the background of insignificant change of flavonoid glycosides content substantial fluctuation of total and specific radioactivity takes place. At the beginning of test isoroifolin, scolimoside and rutin total radioactivity was 1196, 832 and 1026 imp/min respectively.

Further, by 25 hr of exposure without $^{14}CO_2$ rutin and scolimoside total radioactivity (Fig2) reaches its first maximum, where as the highest incorporation of label into isoroifolin is reached by 49 hr. Further decrease of isoroifolin, scolimoside and rutin total radioactivities respectively 2,2 , 2,1 and 3,5 fold, indicates their transformation and filling up of these compounds content at the expense of nonradioactive precursors. By 97 hr of the experiment enough amount of radioactive precursors was formed that caused significant increase of total radioactivity of all three flavonoids. By 121 hr of the experiment an intensive transformation of newly synthesized radioactive isoroifolin, scolimoside and rutin takes place. Analogous change of specific radioactivity of these three flavonoid glycosides was stated. At the beginning of exposure in atmosphere without $^{14}CO_2$ isoroifolin and scolimoside specific radioactivity approximately is two fold higher than of rutin (Fig.3). On the grounds of kinetics data of total and specific radioactivities of flavonoid glycoside in leaves of Georgian lemon the period of their half-life was calculated. It was equal to 30, 55 and 48 hr for isoroifolin, scolimoside and rutin, respectively.

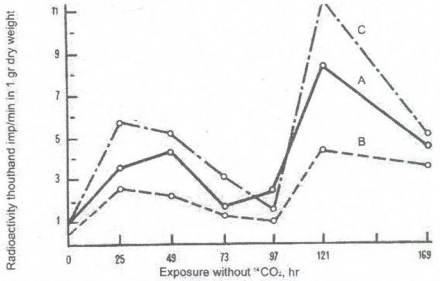

Fig 1. Change of isoroifolin (A), scolimoside (B) and rutin (C) content in leaves of Georgian lemon.

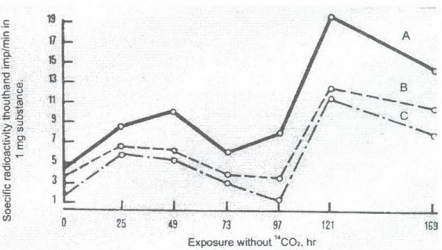

Fig 2. Change of total radioactivity of isoroifolin (A), scolimoside (B) and rutin (C) in leaves of Georgian lemon.

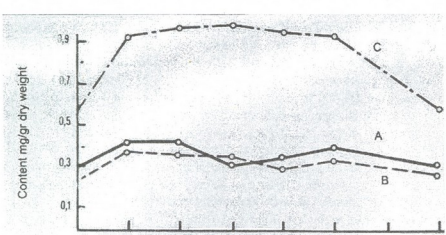

Fig 3. Change of sp. radioactivity of isoroifolin (A), scolimoside (B) and rutin (C) in leaves of Georgian lemon.

Study of metabolic transformations of labeled flavonoid compounds in pumpkin seedlings was shown that on the ground of flavonoid glycoside intensive biosynthesis the period of astragaline, isoquercetin and rutin half-life is 30, 36 and 48 hr, respectively [Strack O., 1976]. In tea plants which is distinguished by active catechin metabolism the period of half-life of (-)-epicatechin and (-)-epigallocatechin is approximately 50 hr [Zaprometov M., 1996]. In seeds of vine tree variety Rkatsiteli an intensive transformation of catechins takes place and the period of half-life of (+)-catechin is 55 hr [Durmishidze S., 1984].

Thus, the results obtained allow to conclude that flavonoid glycosides, such as endogenous isoroifolin, scolimoside and rutin in leaves of Georgia lemon undergo active metabolic transformations.

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ქართული ლიმონის (*Citrus limon Burm.*) ფოთლებში ენდოგენური ფლავონოიდური ნაერთების მეტაბოლიზმის კინეტიკა

თარგამაძე ი., მჟავანაძე ვ., ზამბახიძე ნ., წიკლაური გ., პაპუნიძე ს., შალაშვილი ა.

ს. დურმიშიძის სახელობის ბიოქიმიის და ბიოტექნოლოგიის ინსტიტუტი, საქართველოს მეცნიერებათა აკადემიის

(მიღებულია 10.06.2004)

რეზიუმე

ნაშრომში ნახშირბადის გამოყენებით შესწავლილია ქართული ჯიშის ლიმონის (*Citrus limon Burm.*) ფოთლების ძირითადი ფლავონოიდური გლიკოზიდების იზოროიფოლინის (აპიგენინ-7-რუტინოზიდის), სკოლიმოზიდის (დიუტეოლინ-7-რუტინოზიდის) და რუტინის (კვერცეტინ-3-რუტინოზიდის) მეტაბოლიზმი. ლიმონის მოჭრილ ყლორტებში $^{14}CO_2$ შეყვანილია ფოტოსინთეზის გზით. ექსპოზიციის ხანგრძლივობა შეადგენდა 3 საათს, რის შემდეგ ცდა გრძელდებოდა ბუნებრივი ფოტოპერიოდის - 169 საათის განმავლობაში. იზოროიფოლინის, სკოლიმოზიდის და რუტინის რაოდენობრივი შემცველობის, ჯამური და ხვედრითი რადიოაქტიურობის ცვლილებების მიხედვით ნაჩვენებია, რომ ლიმონის ფოთლებში ისინი განიცდიან ინტენსიურ მეტაბოლურ გარდაქმნებს. ამ ნაერთების ნახევრად დაშლის პერიოდი შეადგენს შესაბამისად 30, 55 და 48 საათს.

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Biotechnology

BIODEGRADATION OF SOME CELLULOSIC MATERIALS BY DIFFERENT TAXONOMIC GROUPS OF MICELIAL FUNGI AND ACTINOMYCETES

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Abstract

Comparative studies of biodegradation of some cellulosic materials - newsprint, filter paper, cardboard and saw dust by using Micromycetes, Basidiomycetes and Actinomycetes have been performed both in solid phase and submerged cultivation conditions. The total cellulase activity of microorganisms was estimated. Conversion of cellulosic substrates to rich with proteins and reducing sugars biomass was observed. Cellulose degradation rate was defined in compliance with concentration of accumulated sugars and reduction of percentage of initial cellulose. Basidiomycetes: *Pleurotus ostreatus 41* and *Lentinus edodes 1000* showed maximum cellulase activity to all above mentioned cellulosic substrates both in solid phase and submerged cultivation conditions. At the same time *Pleurotus ostreatus 41* shows higher growth rate and intensive cellulose biodegradation ability in submerged cultivation conditions compared to other microorganisms.

Key words: Micromycetes, Basidiomycetes, Actinomycetes, Submerged fermentation, Solid phase fermentation, Total cellulase activity.

Introduction

Cellulose, the most abundant organic compound traditionally has been used for energy production. However, in some cases direct combustion of cellulosic materials for energy appears less effective in economics compared to biofuels production especially from cellulosic wastes. A large amount of research on cellulose conversion into glucose that is one of the precursors for production of a wide variety of chemicals and biofuels has been done [Ozkam . et al., 2002; Persiegla G. et al., 2000; Zhao J.et al.,2000] Currently there are two major ways of cellulose converting to glucose: chemical and enzymatic.

Cellulose molecule from various sources are all the same at the molecular level, but they differ in the crystalline structures and bindings to other biomolecules. Bioconversion of cellulose into glucose is performed by cellulase complex that mainly consists of β –endo 1, 4 β glucanase, exo-1, 4 β glucanase and β-glucosidase enzymes. These enzymes are produced by wide variety of fungal and bacterial species. Among the aerobic fungi - *Trichoderma, Fusarium, Aspergilus, Chaetomium* and *Allecheria* are the most active ones resulting in cellulose bioconversion to glucose. The anaerobic bacterial species *Clostridium thermocellum* and *Clostridium*

thermosacharoliticum also represent promising candidates for cellulose conversion to reducing sugars with subsequent production of ethanol [Reith J. et al., 2002].

The overall goal of the proposed research was comparative study of biodegradation of some cellulosic substrates by using different taxonomic groups of mycelial fungi and Actinomycetes. For these purposes the following cellulase producing mycelial fungi and Actinomycetes have been used: Micromycetes - as cellulase producers; Actinomycetes - as amylase and cellulase producers and Basidiomycetes as effective cellulose and lignin decomposers. Cellulose decomposition rate was estimated in compliance with concentration of accumulated reducing sugars and reduction of initial cellulose percentage.

Materials and Methods

Cellulose degradation ability of different taxonomic groups of mycelial fungi, Actinomycetes and Basidiomycetes has been studied both in solid phase and submerged fermentation conditions. All microorganisms listed bellow were delivered by Durmishidze Institute of Biochemistry and Biotechnology.

Two strains of mycelial fungi - Micromycetes*: Aspergilus niger 80* - amylase producer and *Aspergilus versicolor* - cellulase producer have been cultivated at 28^0 C on Chapeck nutrient medium with composition (g/l): $NaNO_3$- 9,1; KH_2PO_4 – 1,0; $MgSO_4$- 0,5; KCl – 0,5; $FeSO_4 \cdot 7H_2O$ – 0,002; pH=5,5.

Two strains of Actinomycetes: *Streptomyces glaukus 71* and *St. Caniferus 82* (isolated from the soil of Georgia) were cultivated at 28^0 C on the following nutrient medium (g/l): KNO_3 - 1,0; KH_2PO_4 - 0,5; $MgSO_4$ – 0,5; NaCl - 0,5; $FeSO_4$ – 0,001; pH=7.0;

Two strains of wood destructing Basidiomycetes*: Pleurotus ostreatus 41* and *Lentinus edodes 1000* were cultivated at 28^0 C on the synthetic medium of the following composition (g/l): Na_2HPO_4 - 0,4; KH_2PO_4 - 0,8; $MgSO_4$ – 0,5; NH_4NO_3 – 3,0; yeast extract – 3,0. 1 ml of 1% solution of microelements: $CaCl_2$, $ZnSO_4$, $CuSO_4 \cdot 7H_2O$ and 2 ml of 5% solution of $FeSO_4$ was added to the nutrient medium. pH was maintained at 5, 5.

Inoculum for Actinomycetes and Basidiomycetes was preliminarily prepared in 750ml cone flasks where the corresponding nutrient medium was introduced in amount of 100 ml. In the flasks for cultivation of Basidiomycetes glucose (10g/l) in addition was introduced as the carbon source. Starch in amount of 20 g/l was used as the carbon source for growth of Actinomycetes. Nutrient media were autoclaved and then small amount of mycelium was introduced there. Incubation of inoculum was performed on the shaker (180 rot/min.) at 28^0C during 7 days.

Comparative studies on biodegradation of newsprint, filter paper, cardboard and saw dust by Micromycetes, Basidiomycetes and Actinomycetes have been conducted both in solid phase and submerged fermentation conditions. At the start of experiments percentage of cellulose in above cellulosic materials was determined.

Under the submerged cultivation 2% of preliminary crumbled up filter paper, newsprint, cardboard and sawdust have been placed in 750 ml volume cone flacks. Nutrient media for mycelial fungi, Actinomycetes and Basidiomycetes in amount of 100 ml were introduced in each flack that were autoclaved at 0,7 atm. for 45 min. After sterilisation of flacks, inoculation of Micromycetes was performed by direct introduction of mycelium taken from the universal solid medium. As for Actinomycetes and Basidiomycetes, incubated inoculum was homogenised and 5 ml of obtained suspension was introduced in the flasks. Submerged cultivation was performed on the shaker (180 rot/min) at 28^0C during 4 days. At the end of fermentation process the flacks' contents were centrifuged at 7000 rot/min. In the supernatant pH and total cellulase activity (TCA) were measured. Percentage of crude protein, cellulose and lignin were determined in the sediment.

Solid phase fermentation of newsprint, filter paper, cardboard and saw dust by mycelial fungi, Actinomycetes and Basidiomycetes was performed in steady-state conditions in thermostat at 28^0 C. 4% of cellulosic substrates were introduced in 100 ml cone flasks. In each flack 13 ml of appropriate nutrient medium was introduced and mixed up to full fluidising of substrate. After sterilization of flacks with cellulosic substrates and nutrient medium, Micromycetes taken from universal solid medium and suspension of Actinomycetes and Basidiomycetes have been individually introduced in the flacks. Duration of experiments was 7 days. At the end of the fermentation process the weight of each flask's contents was determined. 4g of biomass was taken out from each flack and used for determination of percentage of crude protein. The rest amount of biomass was washed out 4 times with 20ml of distilled water, squashed and centrifuged at 7000 rot/min. In the supernatant pH and total cellulase activity was determined. Percentage of crude protein, cellulose and lignin were defined in the biomass rest after the extraction.

Percentage of crude protein in biomass, percentage of cellulose in biomass, lignin, total cellulolitic activity were determined [Termkhitarova, Shulga, 1974; Apdegraph, 1969; Zadrazhili 1977].

Results and Discussion

The aim of proposed research was achievement of maximum biodegradation of some cellulose containing substrates. For these purposes screening of different taxonomic groups of microorganisms such as: Mycelial fungi, Actinomycetes and Basidiomycetes on the filter paper, newsprint, cardboard and sawdust were performed in solid and submerged fermentation conditions. Experiments showed that microorganisms grew on all abovementioned cellulosic substrates and they had cellulose bioconversion ability (Growth rate of microorganisms was estimated in accordance with percentage of crude protein accumulated in biomass).

Table 1. Percentage of crude protein and cellulose in biomass under submerged fermentation process (duration 4 days).

Microorganisms	Crude protein (%)				Reduction of initial cellulose (%)			
	Substrates							
Actinomycetes	Newsprint	Saw dust	Filter paper	Cardboard	Newsprint	Saw dust	Filter paper	Cardboard
St. caniferus K82	2,52	3,36	2,34	2,8	6,68	8,57	36	1,26
St. glaucus K71	4,77	3,08	4,63	3,04	24,36		36,29	7,05
Micromycetes								
Aspergilus niger 80	13,19		10,62	10,48	21,47		42,43	38,4
Aspergilus versicolor	13,61	13,61	13,43	17,26	13,12	14,19	41,86	16,25
Basidiomycetes:								
Pleurotus ostreatus 41	21,24	8,61	16,8	12,16	24,31	13,23	51,91	26,36
Lentinus edodes 1000	18,57	4,53	18,57	5,28	2,78	9,77	46,12	4,72

Table 2. Data obtained under solid phase fermentation conditions (duration 7 days).

Microorganisms	Crude protein (%)				Reduction of initial cellulose (%)			
	Substrates							
Actinomycetes	Newsprint	Saw dust	Filter paper	Cardboard	Newsprint	Saw dust	Filter paper	Cardboard
St. caniferus K82	2,05	2,05	1,12	2,43	6,36	0	7,38	6,08
St. glaucus K71	3,13	2,48	1,82	2,62	11,47	3,18	16,47	5,06
Micromycetes:								
Aspergilus niger 80	2,34	2,43	2,57	4,02	0	0	2,04	8,58
Aspergilus versicolor	2,94	4,21	2,9	3,46	7,72	0	13,52	12,16
Basidiomycetes:								
Pleurotus ostreatus 41	5,24	4,58	3,93	4,39	6,87	2,44	16,7	2,24
Lentinus edodes 1000	7,34	4,53	5,7	4,91	6,98	2,95	17,26	

Results show that under submerged fermentation only *Actinomycetes* give the biomass with low protein content. Table 1 shows that *St. caniferus K82* accumulates crude protein not more than 3, 36%. As for St. *glaucus K71,* it accumulates maximum 4, 77% of crude protein. Basidiomycetes exhibite high growth rate on newsprint and filter paper. *Aspergilus versicolor* known as cellulase producer, grows more rapidly on the cardboard. Comparing the growth ability of microorganisms under submerged and solid phase fermentation conditions (table 2) it could be concluded that submerged fermentation is advantageous. For example, under submerged fermentation conditions Basidiomycetes growing on newsprint produce 3-4 times more crude protein than under solid phase fermentation.

Studies related to biodegradation of different cellulose containing substrates show that all above mentioned microorganisms decompose this complex biopolymer under submerged fermentation. Micromycetes are not able to degrade cellulose when growing on sawdust under solid phase fermentation conditions. Sawdust in general consists of lignin, cellulose and hemi-cellulose and the enzymatic system of Micromycetes can not degrade the cellulose in the complex with lignin, and hemicellulose.

The most effective biodegradation of cellulose was observed under submerged fermentation compared to solid phase fermentation. The maximum cellulose degradation of newsprint was achieved by *St. Glaucus K-71* and *Pleurotus ostreatus 41. Aspergilus versicolor* and *Pleurotus ostreatus 41* are most effectives in sawdust biodegradation. Maximum biodegradation of filter paper was achieved by *Pleurotus ostreatus 41* and *Lentinus edodes 1000. Aspergilus niger 80* and *Pleurotus ostreatus 41* are most active ones in cardboard biodegradation. Taking into consideration that *Pleurotus ostreatus 41* grows rapidly on all above mentioned cellulosic substrates, it can be concluded that this microorganism plays the key role in cellulose biodegradation under submerged fermentation.

During the experimental studies total cellulase activity was determined for all microorganisms (Table 3). Received data show that Basidiomycete - *Lentinus edodes 1000* exhibits the maximum total cellulase activity on the 4th day of submerged fermentation. This microorganism also shows high TCA to sawdust and cardboard under both solid phase and submerged fermentation conditions. The second strain of Basidiomycetes: *Pleurotus ostreatus 41*- revealed high TCA to newsprint and filter paper in solid phase fermentation conditions. At the same time the latter showed high ability for cellulose decomposition.

Table 3. Data of total cellulase activityes.

Total cellulase activity							
Micro organisms:	submerged fermentation (4 days)				solid phase fermentation (7 days)			
	Substrates							
Actinomycetes:	Newsprint	Saw dust	Filter paper	Cardboard	Newsprint	Saw dust	Filter paper	Cardboard
St. caniferus K82	0,003	0,007	0,004	0,002	0	0	0	0
St. glaucus K71	0,004	0		0,005	0	0,006	0	0
Micromycetes:								
Aspergilus niger 80	0	0,001	0,004		0	0	0,006	0
Aspergilus versicolor	0	0	0	0,01	0	0	0	0
Basidiomycetes:								
Pleurotus ostreatus 41	0,003	0,01	0,003	0,02	0,002	0,003	0,02	0
Lentinus edodes 1000	0,009	0,02	0,02	0,04	0	0,01	0	0,006

Based on screening results it can be concluded that Basidiomycetes: *Lentinus edodes 1000* and *Pleurotus ostreatus 41* have the best growth intensity and the highest TCA to different cellulosic substrates. So, these microorganisms can be considered as the promising ones for further studies on pre-treatment of cellulosic wastes resulting in biofuels production.

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ზოგიერთი ცელულოზაშემცველი სუბსტრატების ბიოდეგრადაცია მიცელიალური სოკოებისა და აქტინომიცეტების სხვადასხვა ტაქსონომიური ჯგუფების გამოყენებით

ჩაჩხიანი მ.[1], დუდაური თ.[1], წიკლაური ლ.[1], უგრეხელიძე ვ.[1], ზაქარიაშვილი ნ.[2], ალექსიძე თ.[2], ჯობავა მ.[2]

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რეზიუმე

ზოგიერთი ცელულოზაშემცველი სუბსტრატების მაქსიმალური ბიოდეგრადაციისა და ბიომასაში ხსნადი შაქრების დიდი რაოდენობით დაგროვების მიზნით, მიკროორგანიზმთა განსხვავებული ტაქსონომიური ჯგუფების (აქტინომიცეტების, მიკრომიცეტებისა და ბაზიდიომიცეტების) წარმომადგენლებს შორის ჩატარებულია სკრინინგი ფილტრის, გაზეთის, შუყაოს ქაღალდებზე და ნახერხზე სიღრმული და მყარფაზოვანი კულტივირების პირობებში. შედარებულია ფერმენტაციის მყარფაზოვანი და სიღრული პირობები. ნაჩვენებია ცელულოზაშემცველი სუბსტრატების ცილითა და ხსნადი შაქრებით მდიდარ ბიომასად გარდაქმნის შესაძლებლობა. გამოკვლეულია სუბსტრატების ცელულოზური კომპონენტის ბიოდეგრადაცია და ამ პროცესში მონაწილე ცელულაზური ფერმენტი და საერთო ცელულაზური აქტივობა. როგორც მყარფაზოვანი, ასევე სიღრული კულტივირების პირობებში ოთხივე საკვლევ სუბსტრატზე მაქსიმალური ცელულაზური აქტივობა გამოავლინეს ხისდამშლელმა ბაზიდიალურმა სოკოებმა *Pl.ostreatus* 41 და *Lentinus edodes 1000. Pl.ostreatus 41* ამავე დროს ხასიათდება ცელულოზაშემცველ სუბსტრატებზე ზრდის სწრაფი და სიღრმულ პირობებში ცელულაზური კომპონენტის ბიოდეგრადაციის მაღალი უნარით.

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IDENTIFICATION AND RECEPTION OF PURE CULTURE OF THE CELLULOSE DEGRADING BASIDIOMYCETOUS FUNGI FROM DIVERSE ECOLOGICAL NICHES OF GEORGIA

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Abstract

Samples of 356 cultures of cellulose degrading basidiomycetous fungi has been isolated from different ecological niches of Georgia. Among them 85 strains have been grown as a pure cultures, and 75 – identified. For the identified cultures the influence of temperature and pH of the nutrient medium on biomass has been investigated . Color reactions on the existence of phenoloxidases were tested and the growth coefficient of fungi has been studied.

Key words: basidiomycetous fungi, pure culture, biomass, phenoloxidases

Introduction

Processing of agricultural and industrial wastes is a serious ecological problem of the recent years. On the other hand, this raw material may become a source of many useful substances after biotransformation and biodegradation by means of microorganisms. From this point of view basidiomycetous l fungi draw attention due to their ability to destroy the cellulose [Breene, 1990; Royse, 1992; Lai et al., 1994]. These fungi, utilizing the plant raw material, produce proteins and different bioactive compounds like vitamins, enzymes, organic and amino acids, polysaccharides, manure etc. [Solomko, 1988; Daniliak et al.., 1989; Musilek et al., 1989; Mazur et al., 1996; Oguri et al., 1996; Elisashvili et al., 2003].

As Georgia is known by the wide diversity of its climate and soils, it would be of great interest to study the properties of fungi from the different ecosystems, which reveal diverse physiological and biochemical peculiarities. From this point of view it is important to reveal new interesting forms of these fungi by isolation, identification and studying of less investigated species for further utilization in the desirable practical purposes.

Materials and Methods

Samples of basidiocarps of tested fungi were collected in forests of different regions of Georgia: Kazbegi, Tianeti, Dusheti, Borjomi, Tskneti, Tskvarichamia,Tbilisi and hidden in sterile envelopes. Later the samples were used to obtain a pure culture.

In our experiments the tested fungal samples were purified, and treated by alcohol. Inoculum was taken from the basidiocarps in the sterile conditions and placed in a Petri dish. Mycelium, obtained in this way is genetically identical to the initial sample.

While isolating the pure culture from the basidiospores, the sample was placed on a sterile Petri dish, with hymeneal layer down, and the prints of the spores were taken. The spore dust was placed in sterile water and by means of further dilution the inoculum was obtained for cultivating on a solid nutrient medium.

Composition of the solid nutrient medium was (g/l): a) Na_2HPO_4-0.4, K_2HPO_4-0.8, $MgSO_4 \times 7H_2O$-0.5, NH_4NO_3-2.0, yeast extract-2, maltose -5, glucose-5, agar-20. b) wort-0.5%, agar-20. Media with pH 5.0 - 6.5 were prepared. The temperature of mycelium growth was 27°C.

The fungal growth coefficient (GC) was calculated by the formula: $GC = \frac{dgh}{t}$, g-density of the mycelium(1-rare, 2-medium, 3-dense), d-diameter of the colony, h-height of the colony, t-age, in days. The colony is fast growing, when GC>100, middle growing – GC=50-100, slowly growing - GC<50).

The presence of phenoloxidases was determined by color reactions: laccase was tested by α-naphthol (violet or purple color), peroxidase – with 1% solution of pyrogallol+0.4% H_2O_2(1:1) (orange, brown or orange-red color).

Results and Discussion

Pure cultures of 76 strains of 20 genus of higher basidiomyces (*Pleurotus, Fomes, Coriolus, Pseudotremella, Phellinus, Trametes, Coprinus, Cloeophyllum, Fomitopsis, Panus, Lepista, Schizophyllum, Lentinus, Daedaliea, Omphalotus, Pholiota, Phanerochaete, Ganoderma, Marasmius, Piptoporus*) have been obtained.

The basidiocarps, spores and hyphae were studied morphologicaly. Most of the cultivated fungi developed white and fluffy mycelium with some exclusions. For example representatives of the genera *Panus* and *Marasmius* had skinny and pigmented mycelium, *Pleurotus* and *Pholiota* developed as concentrated circles.

It must be mentioned that during first 2-3 days the mycelium was white, further became pigmented in some genera, e.g. in *Lepista* it got grey color, in *Omphalotus* and *Daedaliea* – light brown. Darkening of the nutrient medium was observed in the genera *Marasmius, Omphalotus, Lepista,* and some representatives of *Pleurotus*.

The dependence of basidiomycetes growth on temperature has been studied (Table 1). Four different temperature regimens – 4°C, 20°C, 27°C and 35°C were taken. 27°C seemed to be the most favorable for basidiomycetes growth (10th day), but at 20°C there also was mentioned a positive result. The representatives of *Piptoporus* developed well at both temperatures, while for *Pseudotremella, Lepista* and *Omphalotus* the optimal growth was reached at 20°C. For the strains of genera *Panus* and *Schizophydum* the optimal were both 27°C and 35°C. Compared with other genera, strains of *Coriolus, Phellinus, Coprinus, Gloeophyllum* and *Pholiota* revealed positive growth, but after 10 days growth wasn't mentioned. The opposite result was observed at 4°C. During 10 days mycelium growth was observed only for 6 genera (Table 1), while for

representatives of other genera (*Omphalotus, Fomitopsis, Trametes*) growth was visible after 15-20 days of cultivation.

The pure cultures of *Pleurotus* were grouped as species of the genus*: P. osrteatus,P. florida, P. tigrinus, P. eryngii,* which diversely depend on temperature and posses different growth coefficients. The highest index was obtained in strains *Schizophyllum* (216) and *Piptoporus* (198) genera, the lowest was in genera *Phelinus* (28) and *Marasmius* (26).

Table 1. Growth of the basidiomycetes at different temperature and pH ("-" means that there was no growth, "+" – medium growth, "++" – intensive growth).

Genus	Temperature				pH of the medium			GC
	4°C	20°C	27°C	35°C	5.0	5.8	6.5	at 27°C
Pleurotus	+	+	++	+	++	+	++	90
Fomes	+	+	++	_	_	+	++	76
Coriolus	_	+	++	+	++	+	-	66
Pseudotremella	_	+	++	_	_	+	++	51
Phelinus	_	+	++	+	_	+	++	28
Trametes	_	+	+	_	_	+	++	54
Coprinus	_	+	++	+	_	+	++	32
Gloeophyllum	_	+	+	+	_	+	++	28
Fomitopsis	_	+	++	_	_	_	++	101
Panus	+	+	+	++	_	++	_	36
Lepista	_	++	+	_	_	++	+	85
Schyzophyllum	_	+	++	++	++	+	++	216
Lentinus	_	+	++	_	++	_	_	46
Daedalea	+	+	++	_	_	+	++	52
Ompholotus	_	++	+	_	++	+	_	72
Pholiota	_	+	++	+	_	++	+	49
Phanerochaete	_	+	+	_	_	_	_	38
Ganoderma	_	+	++	_	_	+	++	47
Marasmius	+	+	+	_	_	+	++	26
Piptoporus	+	++	++	_	+	++	_	198

The influence of pH of the medium on basidiomycetes mycelium growth has been studied. (Table1). Representatives of the genera *Lentinus, Omphalotus* and *Coriolus* revealed effective growth on the medium with pH 5.0. For most of fungi the favorable was solid agar medium with pH 6.5, intermediate was pH 5.8. Data for the genus *Pleurotus* were diverse in different representatives of the genus and need further detail investigations for each species.

Table 2. Color reactions on the presence of phenoloxidases in basidiomycetes

Genus	Laccase	Mn-Peroxidase	Genus	Laccase	Mn-Peroxidase
Pleurotus	+	+	*Lepista*	+	+
Fomes	+	+	*Schyzophyllum*	+	+
Coriolus	+	+	*Lentinus*	+	+
Pseudotremella	+	+	*Daedalea*	_	_
Phoeolinus	+	+	*Omphalotus*	+	+
Trametes	+	+	*Pholiota*	_	+
Coprinus	+	+	*Phanerochaete*	_	+
Gloeophyllum	_	_	*Ganoderma*	+	+
Fomitopsis	_	_	*Marasmius*	_	_
Panus	+	_	*Piptoporus*	+	_

From the Table 2 it is clear that strains of 12 genera revealed positive reactions on the presence of laccase and peroxidase. Strains of the genera *Marasmius, Daedalea, Gloeophyllum* and *Fomitopsis* had negative reaction on both enzymes.

So, it should be suggested that the basidiomycetous fungi of Georgia are promising objects for further investigations, because they reveal diverce biochemical and physiological features.

The collection of the basidiomycetous fungi of Georgia of the Durmishidze Institute of Biochemistry and Biotechnology may serve as an interesting informative material of basidial cultures.

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საქართველოს სხვადასხვა ეკოლოგიური ნიშებიდან გამოყოფილი ხისდამშლელი ბაზიდიალური სოკოების სუფთა კულტურის მიღება და იდენტიფიკაცია

ქაჩლიშვილი ე., წიკლაური ნ., მეტრეველი ე., სონღულაშვილი გ., ასათიანი მ., ქუთათელაძე ლ., ალადაშვილი ნ.

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

(მიღებულია 25.06.2004)

რეზიუმე

საქართველოს ტერიტორიაზე გავრცელებული ხისდამშლელი ბაზიდიალური სოკოების ბიოქიმიისა და ფიზიოლოგიის შესწავლის მიზნით, საქართველოს სხვადასხვა ეკოლოგიური ნიშებიდან აღებულ იქნა 356 კულტურის ნიმუში. მათგან სუფთა კულტურის სახით მიღებულია 85 და იდენტიფიცირებულია 76 შტამი. იდენტიფიცირებულ კულტურათა შორის შესწავლილია ტემპერატურისა და pH-ის გავლენა სოკოს ბიომასის ზრდაზე. ჩატარებულია ფერადი რეაქციები ფენოლოქსიდაზების არსებობაზე. შესწავლილია სოკოების ზრდის კოეფიციენტი.

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Biotechnology

OBTAINING OF PROTEIN – RICH BIOMASS BY COMBINED SUBMERGED CULTIVATION OF *A. TERREUS AT – 490* AND *A. ORYZAE 3 – 9 – 15*

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Abstract

To receive the maximal amount of protein – rich biomass optimal conditions for the combined submerged cultivation of two microscopic fungi – *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490* has been investigated. The possibility of using agricultural and food industry remains as a source of carbon in the nutrient medium was corroborated. It was established that biomass obtained after the combined cultivation of *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490* on cellulose containing plant waste contained 40 – 42% protein, 17 – 19% lipids, 12 – 18% carbohydrates and about 2% of nucleic components.

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

Introduction

Microscopic fungi represent convenient producers for recovering deficit in valuable proteins by microbiological synthesis. Diversely from bacteria and yeast they possess ability to convert different plant remains. Mycelium of fungi contains a lot of proteins which are similar to soybean proteins by composition of some irreplaceable amino acids and is of high biological value. Fungal biomass, analogous to plant oils is also rich of lipids and biologically active compounds [Biljana, et al., 2001, Perez J., et al., 2002, Zervakis G., et al., 2001]. The optimization of cultivation conditions of the mycelia fungi as protein producers, with the purpose of biomass processing for obtaining the separate components, would make possible to attenuate the price of products and use them in the food industry.

Materials and methods

The microscopic fungi – cellulase producer mutant strain *A. terreus AT – 490* and amylase producer mutant strain *A. oryzae 3 – 9 – 15* were taken with the purpose of protein rich biomass producing. These strains were received from the collection of microorganisms of the Institute of Biochemistry and Biotechnology of the Georgian Academy of Sciences.

Optimal temperature of cultivation for *A. terreus AT – 490* is 40°C. This strain was cultivated on the nutrient medium, selected for cellulase biosynthesis (modified medium of Chapek

– Dox): (g/l) - $NaNO_3$ – 3.0, $KH2PO_4$ – 0.2, $MgSO_4 \cdot 7H_2O$ – 0.5, KCL 0.5, $FeSO_4$ - 0.02, maize extract – 25ml; pH of the medium was 4.5.

The optimal temperature for *A. oryzae 3 – 9 – 15* growths is 30°C. This strain was cultivated on the medium, selected for amylase biosynthesis: (g/l) - $NaNO_3$ – 0.9 – 3, $KH2PO_4$ – 2.0, $MgSO_4 \cdot 7H_2O$ – 0.5, KCL 0.5, $FeSO_4$ - 0.02, starch – ml; pH of the medium was 5.8.

In some series of experiments different cellulose containing plant substrates (tea dust, presscake of tomato, citrus flour, maize straw, and vine cuttings) were used as source of carbon in the nutrient medium. Plant substrates were previously dried at 55°C, ground and sieved in 1mm diameter sieve. The treated material was placed in 750 ml volume cone flasks (with amount 5 to 80g/l), one of the above mentioned nutrient mediums was added and sterilized at 0.7 atmospheres for 45 min.

Micromycetes were sowed either simultaneously (in one stage), or in two stages: at the first stage one of the producers was sowed *(A. oryzae 3 – 9 – 15* or *A. terreus AT – 490*), and later another producer was added at the same flask. Mutant strains were cultivated at 30°C and 40°C, on the shaker with 180rot/min. After the cultivation was over, the content of the flasks was centrifuged. The obtained biomass was dried at 55°C and the amount of main components of the biomass was determined: the content of total nitrogen [Konarev V., 1973], humidity, ash elements and lipid content [Burschtein A.,1963], amount of soluble carbohydrates [Maksimenko S., et al.,1975], nucleic acids [Spirin A., 1985].

Results and discussion

As it is known, the accumulation of protein in biomass is greatly influenced by the composition and pH of the nutrient medium, temperature and time of cultivation. As the optimal temperatures of growth for *A. terreus AT – 490* and *A. oryzae 3 – 9 – 15* are different, the initial task was to determine the optimal temperature for combined cultivation of the mutant strains. For this purpose the tested mutant micromycetes were cultivated simultaneously at 30°C, 35°C and 40°C.

The cellulase producer *A. terreus AT – 490* reveals its maximal cellulase activity while cultivated on the maize extract containing medium, and *A. oryzae 3 – 9 – 15* – prefers starch containing nutrient medium. According to these data, we decided to study the influence of mentioned nutrient mediums on the accumulation of proteins in biomass. The duration of cultivation and pH of the medium were also taken into account.

Data of the Table 1 show that the one–stage simultaneous cultivation of the experimental strains inhibited their growth and protein accumulation process in biomass (the maximal content of proteins didn't exceed 16%).

The intensive accumulation of proteins (35 – 40%) was observed in two – stage cultivation of *A. terreus AT – 490* and *A. oryzae 3 – 9 – 15* (Table 2, variants 4 and 10). At the first stage of experiment one of the producers was cultivated on the favorable nutrient medium and optimal temperature for 48h. At the second stage of cultivation the conditions were optimal for the second – later added strain, and the cultivation lasted again 48h (the total duration of the experiment 96h).

According to the results of the this experiment, the investigations have been continued by the following scheme: *A. oryzae 3 – 9 – 15 →A. terreus AT – 490,* when the fermentation is performed in two stages for 96h, following the 30°C→40°C temperature regimen.

The further task was to obtain a protein – rich biomass following the above determined scheme of simultaneous cultivation of A. *oryzae 3 – 9 –15* and *A. terreus AT – 490*, on the various plant substrates. For this purpose the influence of different concentrations of cellulose containing substrates on the protein accumulation process in biomass has been investigated.

Table 1. Influence of cultivating conditions on protein accumulation in biomass during the simultaneous (one - stage) cultivating of *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490*

Variant	Nutrient medium	Temperature, °C	Duration of cultivation, h	Crude protein %
A. terreus AT – 490 + A. oryzae 3 – 9 – 15	For A. terreus AT – 490	30	72	14
A. terreus AT – 490 + *A. oryzae* 3 – 9 – 15	" – "	35	72	14
A. terreus AT – 490 + A. oryzae 3 – 9 – 15	" – "	40	72	16
A. oryzae 3 – 9 – 15 + *A. terreus* AT – 490	For *A. oryzae* 3 – 9 – 15	30	72	12
A. oryzae 3 – 9 – 15 + *A. terreus* AT – 490	" – "	35	72	12
A. oryzae 3 – 9 – 15 + *A. terreus* AT – 490	" – "	40	72	13

Table 2. Influence of cultivating conditions on protein accumulation in biomass during the combined (two - stage) cultivating of *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490*

Variant	Nutrient medium	Temperature, °C	Duration of cultivation, h	Crude protein %
A. oryzae 3 – 9 – 15 + *A. terreus* AT – 490	For *A. oryzae* 3 – 9 – 15	30 30	24 24	13
" – "	" – "	30 35	24 48	20
" – "	" – "	30 40	48 24	25
" – "	" – "	30 40	48 48	40
" – "	" – "	35 35	48 48	16
" – "	" – "	35 40	48 48	18
A. terreus AT – 490 + *A. oryzae* 3 – 9 – 15	For *A. terreus AT – 490*	30 30	48 48	14
" – "	" – "	35 35	48 48	17
" – "	" – "	40 40	48 48	28
" – "	" – "	40 30	48 48	35

Table 3. Influence of plant substrate concentration on protein accumulation in biomass during the combined cultivating of *A. oryzae 3 – 9 – 15* and *A. terreus AT – 490*

Content of substrate(g/l)	Content of crude protein (%)				
	Tea dust	Presscake of tomato	Citrus flour	Maize straw	Vine cuttings
5	20	20	20	19	19
10	21	23	25	20	19
20	25	23	30	29	22
30	28	26	35	42	25
40	30	30	40	32	20
50	35	34	32	25	19
60	30	38	28	19	19
70	30	32	22	16	15
80	27	28	20	16	15

Data presented in Table 3 clear that the best substrates, from the crude protein containing point of view in biomass, are maize straw (in concentration 30g/l) and citrus flour (in concentration 40g/l). The optimal concentration of tea dust was 50g/l (34% protein), for tomato presscake it was 60g/l (30% protein) and for vine cuttings – 30g/l (25% protein).

The chemical composition of biomass, obtained by the simultaneous cultivation of *A. terreus AT – 490* and *A. oryzae 3 – 9 – 15* on these plant substrates is demonstrated in Table 4.

Table 4. The chemical composition of biomasses obtained by means of *A. oryzae 3 – 9 – 15* and *terreus AT – 490* cultivation on cellulose – containing plant substrates

Plant substrate	Humidity (%)	Crude protein (%)	Carbohydrates (%)	Lipids(%)	Nucleic acids (%)	Ash (%)
Tea dust	5	35	10	12	1.5	4
Vine cuttings	5	25	12	14	2.0	5
Maize straw	5	42	20	17	2.0	6
Citrus flour	6	40	18	19	1.6	5
Presscake of tomato	4	38	16	15	1.6	5

As it is clear from this table, the biomasses of fungi contain great amount of biopolymers. Maize straw with high content of carbohydrates (20%) is the best substrate in this case.

Our research makes possible to conclude that maize straw and citrus flour are perspective substrates for the microbial conversion. The combined cultivation of *A. terreus AT – 490* and *A. oryzae 3 – 9 – 15* on these plant raw materials enables us to obtain biomasses with high content of proteins (40 – 42%), carbohydrates (18 – 20%), lipids (17 -- 19%) and nucleic components (about 2%).

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ცილით მდიდარი ბიომასის მიღება *A. TERREUS AT– 490*-ისა და *A. ORYZAE 3–9–15*-ის ერთობლივი სიღრმული კულტივირების პირობებში

მეტრეველი ე., ალადაშვილი ნ., იაშვილი თ., ზაქარიაშვილი ნ., ჯობავა მ., ურუშაძე თ., ხვედელიძე რ., ხოხაშვილი ი.

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რეზიუმე

ცილის მაღალი შემცველობისა და ბიომასის მაქსიმალური გამოსავლის მიღების მიზნით შესწავლილია ორი მიკროსკოპული სოკოს *A. oryzae 3–9–15*-ისა და *A. terreus AT–490*-ის ერთობლივი სიღრმული კულტივირების პირობები. ნაჩვენებია საკვებ არეში ნახშირბადის წყაროდ სოფლის მეურნეობისა და საკონსერვო მრეწველობის ნარჩენების გამოყენების შესაძლებლობა. დადგენილია, რომ ცელულოზაშემცველ მცენარეულ ნარჩენებზე *A. oryzae 3–9–15* -ისა და *A. terreus AT–490*-ის ერთობლივი კულტივირებით მიღებული ბიომასები შეიცავენ 40–42% ცილას, 17–19% ლიპიდებს, 12–18% ნახშირწყლებსა და 2%-მდე ნუკლეინურ კომპონენტებს.

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Cytology

COMPARATIVE STUDY OF HEPATOCYTE TRANSCRIPTIONAL ACTIVITY AT THE EARLY STAGE OF REPARATIVE GROWTH IN PHYLOGENETICALLY DISTANT ORGANISMS

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Abstract

The changes of transcriotional activity in amphibian *(Triturus vitatus)* and fish *(Salmo fario linne)* hepatocytes at the early stage of reparative growth were studied. It has been established, that gene reprogramming in response to proliferative stimulus takes place in liver cells of these organisms, as it is characteristic of rodents. In particular, transcriptional activity peak has been revealed in trout hepatocytes at the 4^{th} hour, and twice in newt liver cells – at the 4^{th} and 6^{th} hours after operation. According to obtained data we suggest, that related to reparative growth the process of gene reprogramming is realized in similar manner in organisms, phylogenetically distant from rodents.

Key words: transcriptional activity, partial hepatectomy, gene reprogramming

Introduction

As it is already known, liver regeneration starts with gene reprogramming [Lodish, 2000; Groenink, 1998]. Based on this, studying processes taking place at the early stage of reparative growth has become particularly important at present. At first, these processes were investigated on the model of rodent liver [Michalopoulos, 2003; Schwabe, 2003; Lambotte, 1997]. It was shown, that transcriptional activity peak of early response genes following partial hepatectomy reveals at the 6^{th} hour after operation and it is directly related to the appearance of mitoses in regenerating liver tissue [Dzidziguri, 1994].

Besides, analysis of literature data reveals, that comparative study of the processes, connected with regeneration at the early stage of reparative growth has not been investigated in phylogenetically distant organisms. At present, it is unknown, whether gene reprogramming and related cell proliferation is realized similarly in organisms being on the different level of development and belonging to the different systematic groups. By our experiments, carried out for the recent years, the similarities of these processes in regenerating liver of tailless amphibian *Rana ridibunda* has been established.

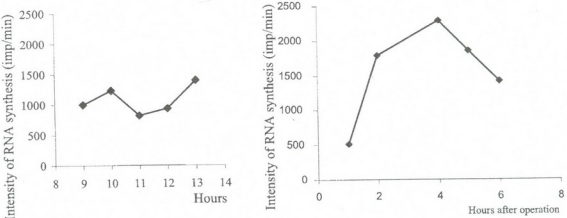
According to all above mentioned, to establish the general principles involved in liver regeneration we aimed to study hepatocyte nuclei transcriptional activity changes at the early stage of reparative growth in organisms phylogenetically distant from rodents (caudate amphibians and fishes).

Materials and methods.

In the experiments were used adult newt (*Triturus vitatus*) and trout (*Salmo fario linne*). Partial resection of liver (1/3 part) was carried out in morning hours. To determine transcriptional activity liver tissue was taken once in an hour during a day. The isolation of nuclei was carried out by Chauveau. DNA quantity in nuclei was determined by Sadovsky and Stern. Transcriptional activity was evaluated by the intensity of C^{14}- UTP uptake. Statistical analysis was performed by Student's test.

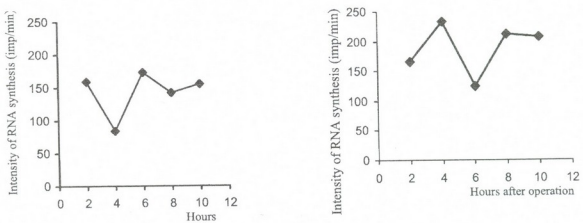
Results and discussion.

In the first series of experiments we have studied transciptional activity changes of intact fish liver cell nuclei during a day. The analyses of obtained data revealed that like other organisms studied earlier, RNA-synthesis in intact fish hepatocytes changes every hour (fig 1, a).



a) b)

Fig 1. The changes of RNA-synthesis activity in trout liver cell nuclei in norm (a) and after partial hepatectomy (b)



a) b)

Fig. 2. The changes of RNA-synthesis activity in newt liver cell nuclei in norm (a) and after partial hepatectomy (b)

As it has been already mentioned, the early stage of reparative growth in rodent (rat and mouse) hepatocytes starts with gene reprogramming. The immediate early gene products expressed within 30 minutes post operation stimulate hepatocyte transcriptional activity [Lodish, 2000; Groenink, 1998]. For that purpose at the next stage of the experiments we carried out partial resection of trout liver.

It should be noted that resection of trout liver (and generally of fish) is connected with some difficulties such as an entirely different localisation of liver which could be cause of so little information about fish liver regeneration. Only the histoarchitectonical changes after partial hepatectomy and hepatotoxin treatment has been described so far. There is also literature data about stem cells, which take part in liver regeneration, as well as in bile duct hyperplasia [Fournie, 2002; Mark, 1999]. Howewer, gene reprogramming related to reparative growth has not been studied at all.

Our experiments revealed that in response to proliferative stimulus transcriptional activity curve characteristic of intact trout hepatocytes changed considerably. Particularly, instead of circadian changes RNA-synthesis activity peak has been revealed at the 4^{th} hour post operation (fig 1, b).

Liver regeneration in newts has been studied better, than in fish. It is established, that the intensity of the processes related to reparative growth reduces on the 20^{th}-25^{th} day post operation and in newts, like in rodents, restoration of liver mass (not of the form) takes place [Sidorova 1996]. We have shown that transcriptional activity in newt liver cells changes every hour (fig. 2,a), hepatocytes stimulated for proliferation RNA-synthesis activity changes considerably. In particular, transcriptional activity peak has been revealed twice – at the 4^{th} and 6^{th} hours after operation (fig. 2, b). Hepatocyte RNA-synthesis activity changes in caudate amphibians at the early stage of reparative growth indicate that gene reprogramming leads to cell proliferation in these organisms.

Based on the obtained data we suggest, that gene reprogramming at the early stage of reparative growth is realized in similar manner in phylogenetically distant organisms (fish and caudate amphibians).

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ალდგენითი ზრდის საწყის ეტაპზე ჰეპატოციტების ტრანსკრიპციული აქტიურობის შედარებითი შესწავლა

გურუშიძე მ., აბრამიძე თ., კლიმიაშვილი თ., ძიძიგური დ.

ციტოლოგიის, ჰისტოლოგიის და განვითარების ბიოლოგიის კათედრა, განვითარების ბიოლოგიის ლაბორატორია, ივ. ჯავახიშვილის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

შესწავლილია უკუდო ამფიბიის (*Triturus vitatus*) და ძვლოვანი თევზის *(Salmo fario linne)* ჰეპატოციტების ტრანსკრიპციული აქტიურობის ცვლილებები ღვიძლის აღდგენითი ზრდის საწყის ეტაპზე. დადგინდა, რომ პროლიფერაციული სტიმულის საპასუხოდ აღნიშნული ორგანიზმების ღვიძლის უჯრედებში, მღრღნელების მსგავსად, ხდება გენების რეპროგრამირება. კერძოდ, კალმახის ჰეპატოციტებში ტრანსკრიპციული აქტიურობის პირველი პიკი ღვიძლის ნაწილობრივი რეზექციიდან მე-4 საათზე ვლინდება, ხოლო ტრიტონის ღვიძლის უჯრედების ბირთვების რნმ-მასინთეზებელი აქტიურობა ორჯერ აღწევს პიკს, ოპერაციიდან მე-4 და მე-6 საათებზე. მიღებული შედეგებიდან გამომდინარეობს, რომ მღრღნელებისაგან ფილოგენეზურად დაშორებულ ორგანიზმების ღვიძლის უჯრედებში რეპარაციულ რეგენერაციასთან დაკავშირებული გენების რეპროგრამირების პროცესი მსგავსი სქემით მიმდინარეობს.

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Cytology

THE SEASONAL FLUCTUATION OF SIGHT RECOVERY AND THE ROLE OF LIGHT FACTOR IN RAT ENDOGENIC RHYTHM SYNCHRONIZATION

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Abstract

The synchronization of processes taking place in white rat brain tissue has been studied at the early stages of postnatal development (up to 30th day from birth). It has been shown that the synchronal occurrence of proliferative and transcriptional activity in brain cells starts within 3 days after eye opening (the 17th day from birth). The seasonal changes in eye opening time have been revealed. The obtained results make us to conclude that the light as an exogenous factor plays a crucial role in formation and synchronization of endogenous biorhythms.

Key words: daily and seasonal rhythms, sight recovery, endogenous rhythm synchronization

Introduction

As we have shown previously the RNA synthesis in white rat liver cells changes rhythmically during a day [Tavdishvili E, et.al;1999]. It has been shown also that in postnatal development (the first 30 days after birth) the transcriptional and mitotic activity in rat liver tissue also fluctuates rhythmically. During the first two weeks of postnatal development these processes occur asynchronically and the signs of synchronization start to show up within 3 days after eyes opening. This fact makes us to suggest that at the early stages of postnatal development the synchronization of endogenous rhythmical processes occurs due to the exogenous light factor (neuro-humoral regulation).

The synchronization of so-called "endogenous" (circadian) rhythms in mammals is connected with the changes in light-dark period and starts when light affects the retina. The nervous impulses then are transferred within the optical nerve from retina to the hypothalamus suprachiasmic nuclei. The increase of light intensity strengthens the neurosecretion of hypothalamus, while its activity rhythm in turn affects the function of hypophysis. The daily rhythm of hypophysis hormone secretion determines the function of other systems of organism [Ashoff U. 1984].

Thus, the main goal of our present research was to study whether the light factor is responsible for the synchronization of transcriptional and mitotic activity in rat brain cells at the early stages of postnatal development

Materials and methods.

The study was held on 300 growing white rats (the first 30 days of postnatal development). The RNA synthesis was studied in test-system of nuclei isolated from brain tissue according to the methods described previously. The cell dissociation method was used for evaluation of mitotic activity [Tavdishvili E. et al; 1999].

To study the seasonal rhythms of eyes opening the rats were watched in winter and summer months. We also studied the effect of light intensity on the eyes opening in winter. For that purpose the newborn rats were divided into two groups. The first group of animals was placed in the natural light conditions, while the second group was kept in the conditions of limited light (in dark boxes with holes through which light rays were getting inside). Both groups of animals were kept in vivarium in normal light conditions.

Results and Discussion.

According to our results the RNA synthesis in rat brain tissue isolated nuclei changes rhythmically during the first 30 days of postnatal development (fig.1).

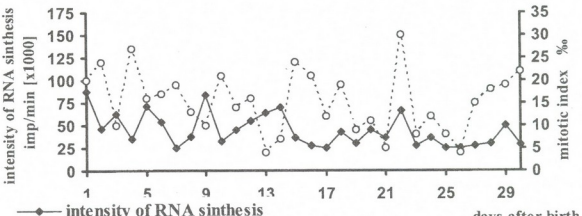

Fig. 1. The changes of RNA synthesis and mitotic activity in rat brain cells during the first 30 days of postnatal development.

The same can be said about the mitotic activity. Beside this, as it can be seen from the figure, on the 3rd day after eye opening (the 17th day after birth) these two processes start to get synchronized. Thus, at the early stages of postnatal development the endogenous processes in brain tissue cells as well as in other types of cells (hepatocytes and splenocytes) start to synchronize at the 3rd day after sight recovery.

It is underlined in the literature that circadian and seasonal rhythms in mammals are determined by the light factor [Oransky I.E., 1988]. For example, such process as spermatogenesis is a seasonal event [Zheleznyac E.V., 2002]. The seasonal biorhythms of hypophysis-thyreoid hormone secretion in females of different age have been also studied (Vasechkina et.al., 2003). The motile activity of rodents also depends on the season of the year – the motion coefficient is higher during spring and autumn and minimal in winter [Valeeva et.al., 2002]. The newborn rats open their eyes on the 14-17th day after birth, but it remained unknown if this process depends on the season. As we have shown in winter months, when the light period is shortened the rats open their

eyes on the 18th day after birth, while in summer the eye opening occurs comparatively earlier – on the 14-15 day after birth. To study whether this process depends on the light intensity and/or light period duration we performed a series of experiments.

Table 1.

Animal group	Light intensity	*Eye-opening time*		
		December	*January*	*February*
I.	Natural light *	18th day	18th day	17th day
II.	Limited light **	18th day	19th day	18th day

*. Natural light means that animals were placed in open cages in vivarium.
**. Limited light means that animals were kept in closed boxes in vivarium.

In the conditions of limited light (II group) the time of eye opening does not change strongly in comparison with the animals kept in natural light (I group) (Table 1.). It seems that the light intensity does not affect the process of sight recovery.

The role of light factor in daily rhythms of different physiological processes (temperature fluctuations, blood circulatory and digestive system activity) corresponded to the rhythms in animal behavior has been studied by many researchers. We can conclude from the literature and our data that light as an exogenous factor plays a very important role in the formation and synchronization of endogenous biorhythms.

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თვალის ახელის სეზონური რხევები და სინათლის როლი ვირთაგვას ენდოგენური რიტმების სინქრონიზაციაში

თავდიშვილი ე.,[1] გაგუა მ.,[2] გოგსაძე ლ.,[1] ძიძიგური დ.[1]

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რეზიუმე

შესწავლილია განვითარების ადრეულ პოსტნატალურ პერიოდში (დაბადებიდან 30 დღის განმავლობაში) თეორი ვირთაგვას თავის ტვინის უჯრედებში მიმდინარე პროცესების სინქრონიზაცია. ნაჩვენებია, რომ უჯრედების გამრავლება და ტრანსკრიპციის პროცესების სინქრონული მიმდინარეობა იწყება თვალის ახელიდან სამი დღის (მე-17 დღე) შემდეგ. დადგენილია თვალის ახელის პროცესზე სეზონური რხევების ზეგავლენა. მიღებული შედეგებიდან გამომდინარეობს, რომ სინათლე, როგორც ეგზოგენური ფაქტორი, განსაზღვრავს ორგანიზმის ენდოგენური ბიორიტმების ჩამოყალიბებასა და სინქრონიზაციას.

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Ecology

THE RARE AND ENDANGERED SPECIES OF THE BLACK SEA BASIN AND THEIR PROTECTION

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Abstract

The preservation of biological diversity is an urgent problem of today. The monitoring of biodiversity is carried out at different levels of organization of life, more often at the level of species. The analysis of biodiversity of aquatic animals of the Black Sea basin and the list of rare and endangered species are given

Key words: Biological diversity, habitats, Mammalia, invertebrate animals

Introduction

Sea–front line of the Black Sea basin represents rich biological diversity. There are 169 species of fish and many other animal species and among them a lot of endems as well as introduced species.

At the end of the XX century pollution of the Black Sea basin was at the critical state, that greatly influenced the living organisms in the sea and intra- reservoirs. As a result, many species left the sea–front line and moved to the Mediterranean Sea and oceans, or died out at all. The degree of these process is not quite clear yet.

So, the main goal of our research was to classify the rare and endangered vertebrates and invertebrate animal species of the Black Sea basin [Mazmanidi, 1991, Mazmanidi and Komakhidze, 1995].

Materials and methods

Material has been collected in 1990-2002 from the waters of south-east part of the Black Sea. In the coastline of Georgia the material has been caught with seabed fishing nets. Monitoring of rare and endangered vertebrate and invertebrate animals in the fish markets of the sea-cost towns of Georgia, Turkey and Russia was also hold. To precise of the list of the rare hydro-bionts Georgian, Russian, Ukrainian, Romanian, Bulgarian and Turkish scientific sources were used [Black Sea Red Data Book, 1999, Komakhidze and Mazmanidi, 1998].

On the basis of these data the rare and endangered species of aquatic animals of the Black Sea were classified according to the contemporary systematization.

Results

On the basis of our and the literature data the full precise list of rare and endangered species was worked out (Table 1)

Table 1. The Annotated list of the rare and endangered species in the Black Sea basin.

	Mammalia
1.	*Monachus monachus* (Hermann, 1779)
2.	*Delphinus delphis* (Linnaeus, 1758)
3.	*Phocoena phocoena* (Linnaeus, 1758)
4.	*Tursiops truncates* (Montagu, 1821)
5.	*Lutra lutra* (Linnaeus, 1758)
	Pisces
1.	*Acipenser güldenstaedti* (Brandt, 1833)
2.	*Acipenser Stellatus* (Palas, 1711)
3.	*Aidoblennius sphynx* (Valenciennes, 1836)
4.	*Belone belone euxini* (Günther, 1899)
5.	*Callionymus belenus* (Risso, 1826)
6.	*Clupoennela cultriventris* (Nordmann, 1840)
7.	*Connger conger* (Linnaeus, 1758)
8.	*Diplodus annularis* (Linnaeus, 1758)
9.	*Gobius buchichi* (Stendachner, 1870)
10.	*Dobius cobitis* (Pallas, 1811)
11.	*Knipowitschia longicaudata* (Kessler, 1877)
12.	*Hippocampus guttulatus microstephanus* (Slastenenko, 1937)
13.	*Lipophrys pavo* (Riso, 1810)
14.	*Liza ramada* (Risso, 1826)
15.	*Lucioperea marina Cuvier* (Valenciennis, 1828)
16.	*Mesogobius batrchacephalus* (Pallas, 1811)
17.	*Neogobius ratan* (Nordmann, 1840)
18.	*Proterorhinus marmaratus* (Pallas, 1811)
19.	*Mullus barbatus ponticus* (Esipob, 1927)
20.	*Nerophis ophidion* (Linnaeus, 1758)
21.	*Pungitius platygaster* (Kessler, 1859)
22.	*Sarda sarda* (Bloch, 1793)
23.	*Scomber scombrus* (Linnaeus, 1758)
24.	*Scorpaena porcus* (Linnaeus, 1758)
25.	*Solea nasuta* (Nordmann, 1840)
26.	*Spicara smaris* (Linnaeus, 1758)
27.	*Symphodus ocellatus* (Forsskal, 1775)
28.	*Symphodus tinca* (Linnaeus, 1758)
29.	*Syognatus tenuirostris* (Linnaeus, 1758)
30.	*Syngnatus typhle* (Linnaeus, 1758)
31.	*Thunnus thunnus* (Linnaeus, 1758)
32.	*Trigla lucerna* (Linnaeus, 1758)
33.	*Vranoscopus scaber* (Linnaeus, 1758)
34.	*Xiphiaaaas gladius* (Linnaeus, 1758)
35.	*Zosterisessor phiocephalus* (Pallas, 1811)
	Invertebrate Animals
1.	*Anat imperator* (Leach, 1915)
2.	*Anomalocera patersoni* (Templeton, 1837)

3.	*Aporrhais pespelecani* (Linnaeus, 1758)
4.	*Apseudopsis ostroumovi* (Bacescu s. Carausu, 1947)
5.	*Biancolina cuniculus* (Stebning, 1874)
6.	*Branchinectella media* (Schmankewitsch, 1873)
7.	*Branchinectella Spinosa* (Schmankewitsch, 1873)
8.	*Branchiostoma lancelatus* (Pallas, 1774)
9.	*Calepteryx splendeus* complex
10.	*Calopteryx Virgo* complex
11.	*Carcinus mediterraneus* (Czerniavsky, 1884)
12.	Centropages Kroyeri pontica (Karawaev, 1895)
13.	*Chaetogammarus ischnis major* (Stebling, 1898)
14.	*Colpocyclops dulcis* (Monchenko, 1977)
15.	*Colpocyclops longispinosus* (Monchenko, 1977)
16.	*Dikerogammarus vilosus* (Sovinskü, 1894)
17.	*Diogenes pygilator* (Roux, 1828)
18.	*Donacilla cornea* (Poli, 1791)
19.	*Epallage fatime* (Charpentier, 1840)
20.	*Eriphia verrucosa* (Forskall, 1755)
21.	*Halacarelus proceerus* (Viets, 1927)
22.	*Halichondria panicea* (Pallas, 1766)
23.	*Hemimysis anomala* (G. O. Sars, 1907)
24.	*Hemimysis serrata* (Bacescy, 1938)
25.	*Hesionides arenarius* (Friedrich, 1936)
26.	*Jphigenella acanthopoda* (G. O. Sars, 1896)
27.	*Jphigenella andrussovi* (G. O. Sars, 1896)
28.	*Jphigenella shablensis* (Carausu, 1943)
29.	*Katamysis warpachowskyi* (G. O. Sars, 1893)
30.	*Macropipus arcuatus* (Leach, 1814)
31.	*Marthasterias glacialis* (Linneaus, 1765)
32.	*Moerisia maeotica* (Ostroumov, 1896)
33	*Oithona minuta* (Kricragin, 1873)
34.	*Ophelia bicornis* (Savigny, 1820)
35.	*Ostrea edulis* (Linneaus, 1758)
36.	*Pachygrapsus marnoratus* (Fabricius, 1787)
37.	*Patella tarentina* (Scalis, 1793)
38.	*Pilumnus hirtellus* (Linneaus, 1758)
39.	*Pontella mediterranea* (Claus, 1863)
40.	*Potamon tauricum* (Cherniavsky, 1884)
41.	*Smirnoviella raducta* (Monchenko, 1977)
42.	*Solen vagina* (Linneaus, 1758)
43.	*Upogebia pusilla* (Peranga, 1792)
44.	*Xantho poressa* (Olivir, 1792)

The list shows that along with vertebrates and fishes there are a lot of invertebrate animals. Among them the majority is crustaceans and mollusks, i.e. animals not capable of active movement on long distances and thus to change the residence. This causes intra-species morphological diversity, increasing the death rate, leading to the extinction of some species.

We consider that it is necessary to state the front-line of the Black Sea basin as "hot spots" of the biodiversity. The most alarming spots are the stone – rock habitats of the front-line,

where the most rare species of epifauna reside. Besides, the sewage and reservoirs near the sea-cost are rich with rare and endangered species as well.

The way out of this situation would forbid fishing of rare and endangered species that will be the pre-condition for their rescuing.

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შავი ზღვის აუზის ობშიათი და გადაშენების პირას მყოფი სახეობების დღევანდელი მდგომარეობა და დაცვის ღონისძიებები

გოგმაჩაძე თ.

ზოოლოგიის და წყალბიორესურსების კათედრა, ბათუმის შ. რუსთაველის სახელობის სახელმწიფო უნივერსიტეტი

(მიღებულია 17.05.2004)

რეზიუმე

შესწავლილი და დახუსტებულია შავი ზღვის აუზის სანაპირო ზოლის ობშიათი და გადაშენების გზაზე მდგარი ძუძუმწოვრების, თევზებისა და უხერხემლო ცხოველების სახეობრივი შემადგენლობა. განსაზღვრულია მათი დღევანდელი მდგომრეობა და მოცემულია დაცვის ღონისძიებები.

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Ecology

SOME BIOMETRICAL PARAMETERS OF THE BLACK SEA ANCHOVY (*ENGRAULUS ENCRASICOLUS L.*) POPULATION AS AN INDICATOR OF BIOLOGICAL POLLUTION OF THE BLACK SEA

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Abstract

During observation period (1990/1991- 1999/2000 seasons) three biometrical parameters of the Black Sea Anchovy (*Engraulus encrasicolus L.*) population hibernated at the Black Sea coast of Georgia were examined: average length and weight, structural staff. At the end of 90-s increase of average length and weight, also enrichment of age spectrum were noted. It was assumed, that at the beginning of 90-s the recession of parameters can be considered as a result of infringement of ecological balance caused by over-catch and pollution of the sea with *Mnemiopsis leidyi.*

Key words: Anchovy (*Engraulus encrasicolus L.*), population, biostatistics, correlation coefficient, regression coefficient.

Introduction

Commercially and ecologically the Black Sea anchovy is a fish species of primary importance in the basin and as the most abundant, it is traditionally the object of fishery in the Black Sea. According to FAO data the fish catch in the Black Sea has fallen from 900 thousand tons (1986) to 100 thousand tons (1992). The great bulk of these catches was anchovy, among them - 40 % were caught at the coast of Georgia. Anchovy catching in the Georgian territorial waters has fallen from 80 thousand tons (70-80s) to 2-7 thousand tons (90s) caused by the economical conditions of the country, reduction of fish stocks and infringement of ecological balance. Brought in the Black Sea basin the invertebrate predator comb-jelly (*Mnemiopsis leidyi*) has negatively affected on anchovy stocks, as the food competitor and annihilator of egg and juvenile.

In the summer anchovy is distributed practically all over the Black Sea. With the temperature changes anchovy migrates to the south of the Black Sea - the Romanian and Bulgarian coastlines, then they reach Turkish Anatolia and even Georgia for wintering. Black Sea anchovy spend the winter near the Georgian coast and in Turkish waters [Chashchin, 1997]. At the coast of Georgia the intensive catching coincided with penetration of *Mnemiopsis Leidyi* and its massive reproduction caused degradation of anchovy population.

The data of two parameters are necessary for performance of a population dynamics: general and biostatistical. Their changes in ecosystem are caused by biotic and abiotic factors. As anchovy in summer period is distributed all over the sea biometrical study of hibernated population enables us to assign ecological conditions.

The aim of this work was to observe some biometrical parameters of the Black Sea Anchovy population hibernated at the coast of Georgia: average length and weight, structural staff. Also, to reveal reason-consequence connection between ecological situation of the see and changes of biostatistical indices of population, comparison and analysis of 10-years data.

A material and methods

In the frames of joint Georgian-Ukrainian research work the materials were received in the Gonio-Kulevi area (depth 15-100м, distance from coast 0,5-1,5 miles). The materials of 76 trawling and 26 purse seine 1000 samples for a season were processed.

After definition of species structure ichthyological test from 1 kg or 100 specimen was made. The selection was carried out representatively.

The primary processing of tests was carried out by individual measurement and it was distributed in various classes (class interval 5 mm). Then the definition of quantity and weight of fishes in given interval was carried out. For definition of age the individuals were taken from classes proportionally. Annual rings, marked on otoliths coincide with annual rings of scales. The annual ring corresponds to the border between internal narrow and external wide rings.

The tests were worked out statistically [Sparre, Venema,1992]. Number of age groups and percentage were established. By "size-age" method (that shows age distribution of fishes of same length and seasonal size structure of age groups; the sums of horizontal and vertical columns of the table are equal) the size-age composition was made [Mayorova,1961].

By linear regressive analysis the correlation between the size and weight of anchovy was revealed. The correlation and regression coefficients were calculated by Microsoft Excel.

Results and Discussion

Anchovy as the species with short life cycle, is characterized by surplus of juvenile. Planktiverous fishes were characterized with legible changes of the quantitative fluctuation and age structures, connected with the changes of fodder supply [Danilevskiy, Mayorova,1979]. All changes in a trophic circuit (plankton – anchovy - predator) are reflected on a quantitative and structural staff of a population.The average size and weight of anchovy during 10 seasons are given in table 1.

The significant parameter of a population is age composition, which influences on its birthrate and mortality. The attitude of various age-grades of populations at present defines its capacity of productivity and demonstrates the future position of a population. The 10-year's structural staff of a population is presented in table 2.

The analysis of a structural staff of anchovy population in 1990/91-1999/2000 fishing seasons allows to conclude that average length and weight were increased from 84,6 mm up to 90,4; 103,2; 92,7 mm and from 4,4 g up to 6,5; 7,8; 6,3 g (Tab.1). Size - weight index in the given decade the most critical was in 1991/92 season, when average length has compounded 78,5 mm, and average weight - 3,6 g. The maximum of parameters were noted in 1998/99 season - 103,2 mm and 7,8g. Variation numbers of last seasons were noted with abundant of class intervals. Age spectrum of a population was getting rich: in first season of observation the age-grade was presented by 4 units, subsequent seasons - 3 units, and in last seasons - 5 units.

Table 1. Average length and weight of Black Sea anchovy

Index	Length (mm)	Average weight (g)									
		1990-91	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98	1998-99	1999-00
		I	II	III	IV	V	VI	VII	VIII	IX	X
1	45-50	-	0,7	-	0,6	-	-	-	-	0,9	-
2	50-55	1,0	1,0	-	1,	1,55	1,7	-	1,5	1,1	1,1
3	55-60	0,9	1,1	-	1,6	1,73	1,87	1	1,5	1,3	1,4
4	60-65	1,4	1,7	2,9	1,9	2,38	2,0	2,7	2,2	1,6	1,7
5	65-70	1,8	2,1	3,8	2,6	2,85	2,94	3,0	2,6	2,1	2,1
6	70-75	2,8	2,7	3,9	3,1	3,2	2,98	3,4	3,0	2,8	2,7
7	75-80	3,9	3,2	3,9	3,8	4,0	3,81	4,1	3,7	3,5	3,4
8	80-85	4,6	3,9	4,0	4,0	4,7	4,16	5,0	4,2	4,0	4,2
9	85-90	5,5	4,6	4,5	4,9	5,65	5,27	5,88	5,3	4,8	5,0
10	90-95	6,7	5,3	4,9	5,0	7,0	5,87	5,80	6,4	5,9	6,1
11	95-100	7,5	6,4	5,9	5,9	7,43	9,5	8,3	7,8	7,0	7,2
12	100-105	9,8	7,2	6,0	7,7	8,37	7,14	8,6	9,0	8,1	7,8
13	105-110	10,4	8,7	6,1	9,0	9,5	8,60	9,7	9,8	9,2	8,5
14	110-115	11,6	9,8	6,2	10,0	9,7	-	11,6	11,0	10,4	10,0
15	115-120	13,8	10,1	6,5	11,9	11,2	-	13,4	11,7	12,0	11,2
16	120-125	14,5	18,5	7,6	12,8		9,85	12,8	13,0	13,2	12,5
17	125-130	17,0	-	-	-	-	-	19,5	14,2	15,1	14,1
18	130-135	-	-	-	-	-	-	-	18,7	18,3	16,0
19	135-140	-	-	-	-	-	-	-	-	19,7	18,0
20	140-145	-	-	-	-	-	-	-	-	22,7	18,0
21	145-150		-	-	-	-	-	-	-	24	-
22	150-155	-	-	-	-	-	-	-	-	-	25
23	155-160	-	-	-	-	-	-	-	-	-	-
24	160-165	-	-	-	-	-	-	-	-	-	32
Average weight, g.		4,4	3,6	5,3	5,0	5,2-	4,8	8,4	6,5	7,8	6,3
Average length, mm.		84,6	78,5	96,5	86,7	82,5	82,4	98,6	90,4	103,2	92,7

Table 2. A structural staff of Black Sea anchovy

N	Catching season	Age group					
		0+	1+	2+	3+	4+	100%
1	1990/91	94,8	3,1	1,0	1,0	-	99,9
2	1991/92	95,0	3,0	1.0	1,0	-	100
3	1992/93	96,1	2,9	1.0	-	-	100
4	1993/94	96,0	3,1	0.9	-	-	100
5	1994/95	77,8	20,2	2.0	-	-	100
6	1995/96	84,3	8,7	7.0	-	-	100
7	1996/97	40,0	50,0	7.8	2,1	-	99,9
8	1997/98	51,5	33,5	7.6	6,5	0,8	99,9
9	1998/99	34,4	33,5	29.7	2,0	0,2	99.9
10	1999/2000	46,3	36,2	12.6	4,3	0,6	100

In a population the percentage 0+ age group was reduced from 94,8 % to 51,5 %, 34,4 %, 46,3 %. The percentage of high age-grades were increased: 1 + age group - from 3.1 % up to 33.5 %, 33.6 % and 36.2 %; 2 + age group - from 1 % up to 7.6 %, 29.7 % and 12.6 %; 3 + age group - from 1 % up to 6.5 %, 2.0 % and 4.3 %.

Despite of downgrade of parameters, the breeds of 1993, 1994 and 1995 have appeared rather fertile. Unlike the previous breed in subsequent catches their introduction is significantly high (diagonal lines, Tab. 2):

a)96,0; 20,2; 7,0; 2,1; 0,8. b)77,8; 8,7; 7,8; 6,5; 0,2. c)84,3; 50,0; 7,6; 2,0; 0,6.

The mass breeding of *Mnemiopsis leidyi* in the beginning of 90-s provoked reduction of a food supply of fishes, which was expressed in weight index, structural and fat content parameters of the Georgian coast anchovy. These parameters were raised at the end of a decade:

1990/91 - 84,6 mm; 4,4 g; 4 units; 10,5 %; 1991/92 - 78,5 mm; 3,6 g; 4 units; 10,0 %;
1997/98 - 90,4 mm; 6,5 g; 5 units; 19,0 %; 1998/99 - 103,2 mm; 7,8 g; 5 units; 13,2 %;
1999/00 - 92,7 mm; 6,3 g; 5 units; 14,9 %.

By the end of 80s the biomass of comb-jelly has reached peak – 1 billion tons [Shuskina, Musaeva, 1990; Shushkina,Vinogradov,1991; Zaitsev, 1995; Prodanov at all., 1996]. The pollution of the sea including mass reproduction of *Mnemiopsis leidyi* caused change in community of plankton. The number of steganopodes and other fodder zooplankton organisms was reduced 15-40 times. In 1991-93 the reduction of invertebrate predators number is noticed. Presumably, the decline of anchovy's parameters in the beginning of 90-s at the coast of Georgia is connected with above-mentioned process. At the coast of Georgia the existence of *Ctenophore Beroe* was stated which is considered as the annihilator of *Mnemiopsis Leidyi*

To establish the correlation between the size L(i) and weight W(i) function of regression was calculated (Fig.1). Regression analyses of 1999/2000 season was expressed by the formula:

$$y=-11,07+1,97x$$

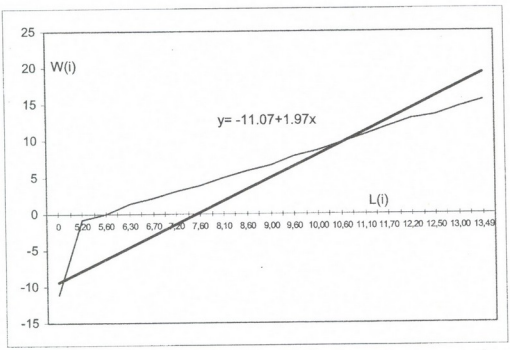

Fig. 1. Anchovy's size-weight dependence regression line

Table 3. Coefficients of correlation and regression of anchovy's size-weight dependence.

Index	Length, cm	Mean weight, g in seasons									
		1990-91	1991-92	1992-93	1993-94	1994-95	1995-96	1996-97	1997-98	1998-99	1999-00
		I	II	III	IV	V	VI	VII	VIII	IX	X
1	5.2	1,0	1,0	-	1,0	1,6	1,7	-	1,5	1,1	1,1
2	5.6	0,9	1,1	-	1,6	1,7	1,9	1.0	1,5	1,3	1,4
3	6.3	1,4	1,7	2,9	1,9	2,4	2,0	2,7	2,2	1,6	1,7
4	6.7	1,8	2,1	3,8	2,6	2,9	2,9	3,0	2,6	2,1	2,1
5	6.2	2,8	2,7	3,9	3,1	3,2	3.0	3,4	3,0	2,8	2,7
6	7.6	3,9	3,2	3,9	3,8	4,0	3,8	4,1	3,7	3,5	3,4
7	8.1	4,6	3,9	4,0	4,0	4,7	4,2	5,0	4,2	4,0	4,2
8	8.6	5,5	4,6	4,5	4,9	5,7	5,3	5,9	5,3	4,8	5,0
9	9.0	6,7	5,3	4,9	5,0	7,0	5,9	5,8	6,4	5,9	6,1
10	9.6	7,5	6,4	5,9	5,9	7,4	9,5	8,3	7,8	7,0	7,2
11	10.0	9,8	7,2	6,0	7,7	8,3	7,1	8,6	9,0	8,1	7,8
12	10.6	10,4	8,7	6,1	9,0	9,5	8,6	9,7	9,8	9,2	8,5
13	11.1	11,6	9,8	6,2	10,0	9,7	-	11,6	11,0	10,4	10,0
14	11.7	13,8	10,1	6,5	11,9	11,2	-	13,4	11,7	12,0	11,2
15	12.2	14,5	18,5	7,6	12,8		9,9	12,8	13,0	13,2	12,5
16	12.5	17,0						19,5	14,2	15,1	14,1
17	13.0		-	-	-	-	-		18,7	18,3	16,0
18	13.5	-	-	-	-	-	-	-		19,7	18,0
Koeficient of correlation		0.983	0.914	0.975	0.976	0.990	0.406	0.955	0.971	0.969	0.977
Koefficient of regression		2.19	1.93	0.70	1.65	1.56	0.59	2.18	1.99	2.18	1.97

Coefficients of correlation and regression of size-weight dependence during 10 season were calculated (Tab. 3):

The values of coefficients of correlation (0,406-0,990) indicates strong connection between weight and size of anchovy. The values of coefficients of regression in 1992/1993 – 1995/96 seasons are low – the increase of length by 1 cm caused the increase of weight by 0,70; 1,65; 1,56; 0,59 g; These values in 1997/1998 – 1999/2000 are high - increase of length by 1 cm caused the increase of weight by 1,99; 2,18; 1,97g.

Thus, according to our data we can conclude that during 1990/91-1999/2000 seasons the values of average length and weights of anchovy were increased. Despite of downgrade of parameters, the breeds of 1993, 1994 and 1995 appeared rather fertile. Unlike the previous breed in subsequent catches their introduction is significantly high, i.e. in 1997/98-1999/2000 seasons age spectrum was getting rich. At the end of 90s increase of percentage of high-age groups was noted. Changes of a structural staff of a population, the increase of the parameters at the last seasons, standardization of fatty depot (13.2-14.9%), restoration of the habitus indicates improvement of population circumstances, anchovy's physiological state and in general environmental conditions.

It is possible to assume, that at beginning of 90s the recession of anchovy's biometrical parameters at the coast of Georgia can be considered as a result of infringement of ecological balance caused by over-catch and pollution of the sea.

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შავი ზღვის ანჩოუსის (*Engraulus encrasicolus L.*) პოპულაციის ბიომეტრული მაჩვენებლები, როგორც შავი ზღვის ბიოლოგიური დაბინძურების ინდიკატორი

შაველაყაძე მ.

ზღვის ეკოლოგიისა და თევზის მეურნეობის სამეცნიერო-კვლევითი ინსტიტუტი

(მიღებულია 07.06.2004)

რეზიუმე

1990/91-200/2001 წლების სეზონებზე მიმდინარეობდა დაკვირვება შავი ზღვის საქართველოს სანაპიროსთან მოზამთრე შავი ზღვის ანჩოუსის პოპულაციის სამ ბიომეტრულ პარამეტრზე: საშუალო ზომა-წონასა და სტრუქტურულ შემადგენლობაზე. 90-იანი წლების დასასრულისათვის შეიმჩნეოდა საშუალო ზომისა და წონის მაჩვენებელთა მატება, ასევე ასაკობრივი ჯგუფების სპექტრული გამდიდრება. შესაძლოა დავასკვნათ, რომ 90-იანი წლების დასაწყისისათვის აღნიშნულ პარამეტრთა შემცირება ჭარბჭერითა და სავარცხლურათი (*Mnemiopsis leidyi*) დაბინძურებით გამოწვეული ზღვის ეკოლოგიური წონასწორობის რღვევის შედეგია.

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Ecology

THE IMPACT OF THE ENVIRONMENTAL POLLUTION ON ANATOMICAL STRUCTURE OF SHRUB LEAVES

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Abstract

The impact of technogenic factors on leaf's anatomical structure of xerophyte shrub plants - *Buxus sempervirens, Laurus nobilis, Spartium junceum, Nerium oleander* was studied. The amount of epidermal cells per unit area of leaf and number of stoma of plants from polluted area are slightly increased compared to the ones from ecological pure area. The thickness of cuticle is increased in comparatively more degree. Mesophyll thickness grows mainly on the expense of palisade parenchyma. *Buxus sempervirens* and *Laurus nobilis* are more affected by pollution than *Spartium junceum* and *Nerium oleander*. As the changes of leaf structure are insignificant all four species can be considered to be resistant.

Key words: environmental pollution, leaf, xerophyte shrubs.

Introduction

Modern biosphere is in the state of ecological crisis. Rapid progress of science and technology negatively affects the environment. The main sources of profound effect on the environment are everyday remains and waste products, dust particles in the atmosphere, emission of various industrial gases, fumes, chemicals used in the agriculture, radioactivity, etc.

Plants are the major source for purifying the atmosphere from technogenic factors. The pollution affects the plants in different degree. To reveal the plants which are resistant to technogenic factors and used for greenery of towns has great practical significance. The impact of technogenic pollution mostly is reflected on leaf anatomy. The effect of pollution is manifested in morphological, anatomical and physiological variations taking place in a leaf. Anatomical changes are expressed in the growth of the thickness of leaf blade, decrease of cells size, increase of the density of their arrangement, number per unit area, cuticle thickness, number of stomas, the degree of palisade parenchyma development in mesophyll [Arsenieva, Chavchavadze, 2001; Gabunia, Tutberidze, 1998; Davbish, 1987; Voron, 1986; Shilov, 1997].

Materials and methods.

The experimental material was taken both from polluted (large industrial environs of the town) and ecologically pure area (resort Sairme, Botanical gardens of Kutaisi). Plants taken from the polluted areas are conventionally called experimental and those from ecologically pure areas –

control. We have studied four plant species: *Buxus sempervirens, Laurus nobilis, Spartium junceum, Nerium oleander.*

Both in experimental and control plants mesophyll and epidermal structure of a leaf have been studied. Cuticle thickness and shape, size of epidermis cells, type of stoma and quantity per unit area, mesophyll thickness and differentiation rate, palisade and spongy parenchyma structures were studied. The leaf's anatomical structure was carried out using the methods offered by Aneli [Aneli, 1975].

Results and Discussion

According to the data of some researchers [Gabunia, Tutberidze, 1998; Davbysh, 1987] the changes caused by air pollution strengthens the features characteristic to xerophilous plants. To reveal what changes xerophilous plant undergoes in such environment, in greenery widely used xerophyte shrubs have been studied .

Under the action of harmful technogenic factors quantitative indices of structural changes taking place in the leaves of the researched plants are given in the Table 1. The differences between the experimental and control variants of the researched plants are insignificant. It should be noted that the researched plants don't respond to the environment pollution in a similar way. In the experimental variants compared with control the amount of epithelial cells per unit area is slightly increased (in *Laurus nobilis*–7.3%, *Buxus sempervirens – 3.7%, Nerium oleander* – 2.5%, *Spartium junceum – 1.6%).* The amount of stomas per unit area caused by pollution is also insignificantly increased. In *Laurus nobilis* it makes 2.5%, in *Buxus sempervirens* – 4.7% and in *Spartium junceum* – 2.5%. As to *Nerium oleander* the amount of stomas per unit area both in control and experimental variants is the same. The cuticle thickness varies in comparatively more degree. Particularly, compared with control cuticle thickness in experimental variants is increased; in *Buxus sempervirens* - 31%, in *Laurus nobilis* - 40%, in *Spartium junceum* -15%, in *Nerium oleander* -13%.

The epidermis of the researched plant leaves is usually one-layer and *Nerium oleander*'s leaf is covered on both sides with multi-layer (three-layer) epidermis. In the leaves of investigated plants the stoma is located on the level of epidermal cells, an exception is *Nerium oleander* which stoma is in leaf's depth, in special hollows, the so-called crypts and this crypt is covered with trichomes from the outside. In *Laurus nobilis* leafs epidermal cells drops of essential oil are found. In experimental variant their amount is abundant. In the epidermis of *Spartium junceum* there are crystals of prismatic and cubic form of mineral substances which amount is increased in experimental variant.

Technogenic factors affect leaf's mesophyll too. According to our data [Gabunia, Tutberidze, 1998] in woody plant leaves the thickness of mesophyll grows under the action of technogenic factors. An increase of mesophyll thickness mainly happens on the expense of palisade parenchyma. From this viewpoint the difference between the experimental and control variants is comparatively more in *Laurus nobilis* and *Buxus sempervirens* and it is less in *Spartium junceum* and *Nerium oleander.*

The obtained data evidence that harmful technogenic wastes negative affect on the inner structure of leaves of the researched plants - *Buxus sempervirens, Laurus nobilis, Spartium junceum* and *Nerium oleander.* In experimental variants of all the researched plants cuticle thickness is increased in comparatively more degree. The amount of epidermis cells and stoma per unit area is little increased, as well as mesophyll and correspondingly palisade parenchyma thickness.

Table 1. Structural Changes caused by technogenic environmental pollution in shrub leaves

Plant	Object	Leaf thickness, µm	Mesophyll thickness, µm	Palisade parenchyma thickness, µm	Spongy paren-chyma	Number of palisade parenchyma layers in mesophyll	Epidermis thickness, µm	Cuticle thickness, µm	Shape of epidermis cells	Configura-tion of epidermis cells coat	Number of epidermis cells per 1 mm^2	Number of stomas per 1 mm^2
Boxus sempervirens	Experiment	218±4.7	178±3.5	75±1.5	123±2.6	2	20±0.4 16±0.3	25±0.4 25±0.3	multiangular multiangular	Linear Linear	5010±200 5235±200	- 155±2.5
	Control	175±3.0	163±2.1	69±1.3	100±2.1	2	16±0.3 16±0.3	19±0.3 19±0.3	multiangular multiangular	Linear Linear	4900±192 5037±200	- 148±2.5
Laurus nobilis	Experiment	192±4.1	117±2.4	45±1.1	71±1.5	1	15±0.2 13±0.2	14±0.2 14±0.2	Irregular Irregular	Curved Curved	3250±170 3917±170	- 120±2.3
	Control	172±3.7	109±1.0	40±1.0	69±1.3	1	13±0.2 12±0.2	10±0.1 10±0.1	Irregular Irregular	Curved Curved	3173±165 3631±165	- 117±2.3
Spartium junceum	Experiment	370±4.6	320±5.2	162±3.1	158±3.0	3	22±0.4 20±0.4	23±0.3 23±0.2	Irregular Irregular	Little waved	4800±181 5860±175	260±30 287±3.2
	Control	352±4.1	310±5.2	158±3.0	152±3.0	3	22±0.4 20±0.4	20±0.2 20±0.2	Irregular Irregular	Little waved	5690±180 5860±175	260±3.0 280±3.2
Nerium oleander	Experiment	390±4.9	340±5.3	130±2.4	210±3.5	3	48±0.8 42±0.7	25±0.4 25±0.4	multiangular multiangular	Linear Linear	4593±181 4791±191	- 95±2.3
	Control	377±4.7	325±5.0	128±2.6	215±3.5	3	48±0.8 42±0.7	22±0.4 22±0.4	multiangular multiangular	Linear Linear	9636±173 4671±170	- 95±2.3

According to the obtained data in conditions of West Georgia all the researched plans can be considered resistant. Among them *Nerium oleander* is distinguished. Multi-layer epidermis on the leaf surface of this plant and deeply located stoma play a certain role of barrier promoting the penetration of toxic substances into the leaf.

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გარემოს ტექნოგენური დაბინძურების გავლენა ზოგიერთი ბუჩქოვანი მცენარის ფოთლის ანატომიურ აგებულებაზე

თუთბერიძე რ., მარგველაშვილი ნ., გაბუნია მ.

ა. წერეთლის სახ. ქუთაისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.06.2004)

რეზიუმე

შესწავლილია ტექნოგენური ფაქტორების გავლენა ზოგიერთი ქსეროფიტი ბუჩქოვანი მცენარის - ბზის, დაფნის, კურდღლის ცოცხასა და ოლეანდერის (*Buxus sempervirens, Laurus nobilis, Spartium junceum, Nerium oleander)* ფოთლის ანატომიურ აგებულებაზე. საკონტროლოსთან შედარებით საცდელ ვარიანტში ოდნავ მომატებულია ფართობის ერთეულზე ეპიდერმისის უჯრედების რაოდენობა, ბაგეების რიცხვი. შედარებით მეტად იზრდება კუტიკულის სისქე. მატულობს მეზოფილის სისქე, ძირითადად მესრისებური პარენქიმის ხარჯზე. გარემოს დაბინძურების გავლენით მომხდარი ცვლილებები შედარებით მეტია დაფნასა და ბზაში და ნაკლები – კურდღლის ცოცხასა და ოლეანდერში. ფოთლის სტრუქტურაში მომხდარი ცვლილებები იმდენად უმნიშვნელოა, რომ ოთხივე სახეობა რეზისტენტულ ფორმებად შეიძლება ჩაითვალოს.

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Genetics

POLYMORPHISM OF C-HETEROCHROMATIN IN CULTURED LYMPHOCYTES FROM PATIENTS WITH SYSTEMIC AUTOIMMUNE DISORDERS

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Abstract

Polymorphisms of chromosomes 1, 9 and 16 were studied by C-banding in patients with systemic connective tissue diseases. According to the summarized data the patients with lupus erythematosus were found to have higher number of enlarged C-bands for chromosome 9 ($\chi^2_3 = 8.3$, $p<0.05$), but not for chromosomes 1 and 16. In the patients with rheumatoid arthritis high tendency to enlargement was revealed for chromosome 9qh, while C-segments on the other two pairs of chromosomes remained stable. No centromeric heterochromatin variability was seen in the patient with scleroderma, where the mean centromeric heterochromatin length of all the three pairs of chromosomes did not differ from that of the control.

Key words: lupus erythematosus; rhematoid arthritis; scleroderma; centromeric heterochromatin; polymorphism

Introduction

Lupus erythematosus is a chronic inflammatory disease affecting all organ systems. Genetic, environmental and hormonal factors play a role in its etiology. Rheumatoid arthritis, a systemic inflammatory disease of connective tissue that progressively affect joints, represents a multifactorial disease with a significant genetic component, while the systemic scleroderma is a truly collagenic disorder of acquired character with surplus formation of immature collagenic fibres. All the three disorders have the autoimmune character with different degrees of severity. On cell level lowered ability to repair gamma irradiation-induced DNA strand lesions and failing in reparative system, on the whole, against the background of genome instability have been stated [Tuschi et al., 1984; Dvalishvili et al., 1999; Sigua et al., 1999]. Deterioration of DNA repair expresses in lowered repair abilities of cells at a senile age [Lezhava, 1999], as well as at a number of diseases [Mikhelson, 1986] are due to chromosome modifications, in particular, progressive heterochromatinization of chromosome regions resulting in inactivation of genes located there [Lezhava, 2001]. In this point of view, study of conformational changes of chromosomes at various pathologies would lead us to better understanding of the mechanisms of any disease.

Results of the investigation of centromeric heterochromatin in cultured lymphocytes obtained from the patients with systemic connective tissue disorders are presented. Comparative analysis of C-band patterns has been performed for three pairs of chromosomes – 1, 9 and 16.

Materials and methods

Blood samples of 10 patients with connective tissue disorders (5 individuals were lupus patients, 4-with rheumatoid arthritis and 1-with scleroderma) and 5 healthy donors (of the middle age) were cultured for cytogenetic analysis and the metaphase chromosomes were subjected to banding treatment according to the slightly modified usual method. 20 cells for each examined individual were analyzed. Centromeric heterochromatin lengths were measured on the 1st, 9th and 16th chromosomes and the short arm of chromosome 16 was used to correct between-cell degrees of chromosome contraction [Patil and Lubs, 1977]. According to their sizes the C-segments were distributed to 5 variants - from the smallest *a* to the largest *e*. χ^2 values for statistical analysis were estimated.

Results and Discussion

The recurrence of constitutive heterochromatin sizes has been assessed in C-bands of chromosomes 1, 9 and 16. The total C band content detected on three studied chromosome pairs have some tendency to increase in patients with systemic lupus erythematosus (χ = 6.31), but not in patients with rheumatoid arthritis or scleroderma. However, the variability of total C-band amount in lupus patients was not statistically significant. The next step in our investigations was to observe heterogeneity of centromeric heterochromatin separately in each of the three chromosome pairs.

Comparative characteristics of chromosomes 1, 9 and 16 C-band distribution are given in Table 1 (for the patients with lupus), Table 2 (for rheumatoid arthritis) and Table 3 (for scleroderma), where: v reflects the numbers of *a, b, c, d* or *e* variants for the cells of control subjects; μ – number of *a, b, c* ...variants for the cells of affected patients; n – total number of C-segments in the cells of control subjects; m – total number of C-segments in the cells of affected patients.

Table 1. C-band polymorphism for chromosomes 1,9 and 16 in patients with Systemic Lupus Erythematosus.

Chromosomes	C-segment variants	v_i	μ_i	v_i/n	$\frac{v_i+\mu_i}{n=m}$	χ^2
1	a	17	16	0.0867	0.0838	1.95 P>0.05
	b	79	80	0.4031	0.4036	
	c	80	73	0.4082	0.3883	
	d	20	29	0.102	0.1244	
9	a	39	43	0.2063	0.2187	8.3 P<0.05
	b	99	75	0.5238	0.464	
	c	50	62	0.2646	0.2987	
	d	1	6	0.0053	0.0187	
16	a	89	95	0.4709	0.4855	1.96 P>0.05
	b	86	75	0.455	0.4248	
	c	14	20	0.0741	0.0897	
	d	0	0	0	0	

Table 2. C-band polymorphism for chromosomes 1,9 and 16 in patients with Rheumatoid Arthritis.

Chromosomes	C-segment variants	ν_i	μ_i	ν_i/n	$\frac{\nu_i+\mu_i}{n=m}$	χ^2
1	a	15	21	0.0962	0.1146	1.92 P>0.05
	b	61	53	0.391	0.3631	
	c	62	62	0.3974	0.3949	
	d	18	22	0.1154	0.1274	
9	a	31	41	0.2039	0.2376	4.21 P<0.05
	b	78	66	0.5132	0.4752	
	c	42	40	0.2763	0.2706	
	d	1	4	0.0066	0.0165	
16	a	72	71	0.4675	0.4703	0.46 P>0.05
	b	70	64	0.4545	0.4408	
	c	12	15	0.0779	0.0888	
	d	0	0	0	0	

Table 3. C-band polymorphism for chromosomes in the Scleroderma patient.

Chromosomes	C-segment variants	ν_i	μ_i	ν_i/n	$\frac{\nu_i+\mu_i}{n=m}$	χ^2
1	a	5	4	0.1125	0.1125	0.65 P>0.05
	b	16	16	0.4	0.4	
	c	16	15	0.4	0.3875	
	d	3	5	0.075	0.1	
9	a	10	10	0.25	0.2597	0.86 P<0.05
	b	18	13	0.45	0.4026	
	c	11	13	0.275	0.3117	
	d	1	1	0.025	0.0259	
16	a	21	14	0.525	0.4545	3.39 P>0.05
	b	19	21	0.475	0.5195	
	c	0	2	0	0.0259	
	d	0	0	0	0	

As it is obvious from the Tables, the extremely large *e* variants of C-bands, that correspond to the double length of the short arm of chromosome16 (2x16p) were not registered neither in affected individuals, nor in control group of donors.

The comparative analysis of the results shows that heteromorphism towards increase of the size of the C-band on chromosome 9 was significantly more common in patients with lupus erythematosus ($\chi^2_3 = 8.3$; $p<0.05$). The *d* variants on chromosome 9 (corresponding to the 1.5 - 2 x 16p) in lupus patients, who were heteromorphic for C-band size were registered in 3% of cases (control value – 0.5%). In chromosome 1 *d* variants were relatively common in both - affected and healthy groups of individuals (total incidence - 14.5% and 10%, respectively). Chromosome 16 contained only small variants of C-bands.

In the patients suffering from rheumatoid arthritis some tendency to the increase of the length of chromosome 9qh was revealed, however the frequencies for C-band size classes in total did not significantly differ from control values.

No variability of centromeric heterochromatin was observed in the patient with scleroderma with a high incidence of smaller C-bands found there.

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C-ჰეტეროქრომატინის პოლიმორფიზმი სისტემური აუტოიმუნური დაავადებების მქონე ინდივიდთა კულტივირებულ ლიმფოციტებში

დვალიშვილი ნ., სიგუა ნ., ბაბლიშვილი ნ., ლომინაძე რ.

გენეტიკის კათედრა, ივ. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

სისტემური შემაერთებელქსოვილოვანი დაავადების მქონე ინდივიდებში C-ბენდირების მეთოდის გამოყენებით შესწავლილია 1-ელი, მე-9 და მე-16 ქრომოსომების პოლიმორფიზმი. სუმარული მაჩვენებლების მიხედვით, სისტემური წითელი მგლურით დაავადებულთა უჯრედებში გამოვლენილია დიდი ზომის C-სეგმენტების მაღალი სიხშირე მე-9 ქრომოსომაზე ($x^2 = 8.3$, $p<0.05$). ეს მაჩვენებელი არ აღმოჩნდა ვარიაბელური 1-ელ და მე-9 ქრომოსომებში. რევმატოიდული ართრიტით დაავადებულ პაციენტთა უჯრედებშიც მხოლოდ მე-9 ქრომოსომის მიმართ აღინიშნებოდა ცენტრომერული ჰეტეროქრომატინის ზრდის ერთგვარი ტენდენცია. სკლეროდერმიით დაავადებული ინდივიდის სამივე წყვილი ქრომოსომის ცენტრომერული ჰეტეროქრომატული რაიონები არავარიაბელური აღმოჩნდა და მათი სიგრძის საშუალო მაჩვენებლები არ განსხვავდებოდა საკონტროლო ჯგუფის მაჩვენებლებისაგან.

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Genetics

DETERMINING OF CHROMOSOME INSTABILITY IN CASE OF DIFFERENTIATED AND UNDIFFERENTIATED FORMS OF OLYGOPHRENIA

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Abstract

The variability of chromosomes has been studied under the influence of 5-bromodeoxiuridine and metatrexat in patients with Down syndrome and undifferentiated forms of olygophrenia. The increased level of general genome instability has been revealed. The cells derived from patients with Down syndrome and undifferentiated form of olygophrenia are characterized with different sensitivity to the harmful effect of the agents.

Key words: Down syndrome, olygophrenia, chromosome, fragile sites.

Introduction

In recent years complex clinical genetic investigations are more rarely used towards certain disorders. At first it deals with the diseases, clinical identification of which is difficult or they are characterized with illegible etiology. A great number of scientific data indicates to the genome instability in case of some tumors [Nesina et al., 2000], certain systemic diseases [Sigua et al., 1999; Lezhava, Bablishvili, 2003] including Hypermobility Syndrome of joints [Dvalishvili et al., 1999] and disorders of thyroid gland [Zosidze, Koplatadze, 2003].

Differentiated forms of olygophrenia compose only half of common psychophysical abnormalities identifed genetically. The other cases are distributed in the group of undifferentiated forms of olygophrenia due to the luck of specific clinical signs or their etiology requires more accurate definition. On the base of our cytogenetic investigations of undifferentiated forms of olygophrenia the group characterized with increased frequency of structural and quantitative chromosomal aberrations has been revealed [Dadunashvili et al., 2003] . In recent years in addition to the chromosome aberration studies, the studies on exhibition and registration of chromosome fragile sites in order to characterize genome instability in a pathological state are also widely carried out. These sites are revealed not only on the autosomes but on the sex chromosomes as well under the specific influence [Kadotani, Vatarabe, 1998]. Martin Bell's syndrome, one of the differentiated forms of olygophrenia, is associated with the fragile X chromosome.

The main goal of our research was to define the frequency of chromosomal fragile sites in peripheral blood lymphocytes of mentally retarded individuals affected with both differentiated (Down syndrome) and undifferentiated forms of olygophrenia.

Material and Methods

The lymphocyte cultures have been derived from the mental retarded adults. The cultivation and harvesting of lymphocytes were carried out using the standard method. To express the fragile sites of chromosomes the cultures were treated with two different agents. The agents – bromodeoxiuridine (BDU) with concentration 20 mg/l and metatrexat (10mg/l), were added to the cultures for 24 hours. BDU, the analogue of thymine, easily displaces it in DNA molecule and therefore causes the incorrect base pairing. BDU represents the universal compound against the induction of side-fragility. Metatrexat is the antagonist of folic acid. It doesn't block the growth of culture in 10mg/l concentration. The frequency of fragile sites induced by metatrexat is significantly depended on the duration of its influence.

The chromosome slides were stained with gimza and the frequency of chromosomal aberrations and gaps were registered.

Results and discussion

The analysis of spontaneous level of chromosome aberrations showed that the average frequencies of aberrant cells in the individuals with Down syndrome and undifferentiated forms of olygophrenia were equal to 4,5±1,4% and 4,4±1,4%, respectively and significantly exceeded the same index of healthy individuals (1,4±0,52%). Both BDU and metatrexat used in order to express the fragile sites as expected revealed sharply depicted clastogonic effect in case of both forms of olygophrenia. However, according to this index, the appropriate specificity was noticed. The chromosomes derived from Down syndrome patients and from the individuals with undifferentiated forms of olygophrenia revealed the distinct behaviour under the influence of the mentioned compounds. The patients with Down syndrome were more sensitive to the harmful activity of BDU (the frequency of chromosomal aberration-containing cells induced by BDU was 33,0±5,2% while in case of metatrexat - 23,0±4,2%); the frequency of aberrant cells in case of undifferentiated forms of olygophrenia reached the maximal level under the influence of metatrexat (the frequency of aberrant cells equaled 24,0±3,0%, in case of BDU – 18,0±2,7%) (Fig. 1).

As for the control group the isolated lymphocyte cultures were influenced only by BDU. In this case BDU in tested concentration induced significant increase in chromosomal aberrations (the frequency of aberrant cells – 8,0±1,9 %), i.e. the individuals from the control group were less sensitive towards the harmful effect of BDU, than the individuals with olygophrenia.

The spectra of chromosomal aberrations in both cases of olygophrenia and in the control group as well, were mainly represented with single and pair fragments. Chromosome and chromatid translocations were rarely registered.

To determine the fragility of chromosome sites the stimulators – BDU and metatrex – were added to the cultures for 24 hours. This duration engenders the maximum effect of metatrexat, while in case of BDU, after 24 hour exposure rare fragile sites are induced [Nesina et al., 2000]. The site-fragility was determined by the registration of chromosomal gaps. The gaps are considered to be the result of DNA decondensation due to the lack of compact structure of metaphase chromosomes.

The analysis of fragile sites revealed that their frequency was significantly increased in case of Down syndrome and undifferentiated forms of olygophrenia in comparison with the healthy controls (0,4 gaps/cell; in control – 0,014 gaps/cell).

The study of fragile sites induced by BDU revealed, that their frequency sharply increases in both forms of olygophrenia compared with the control group. Metatrexat also induces the increase in frequency of gaps. According to this index the difference between Down syndrome and undifferentiated forms of olygophrenia was identified. The individuals with Down syndrome were

more sensitive than the patients with undifferentiated form of olygophrenia (the number of gaps in Down syndrome under the influence of BDU and metatrexat was 0,28 and 0,26 respectively, while in controls – 0,15) i. e. the effects of BDU and metatrexat were approximately the same. The activities of these compounds in case of undifferentiated form of olygophrenia were equal as well (the mean value – 0,21 gaps/cell), however relatively less than in case of Down syndrome (Fig. 2).

As for the distribution of fragile sites throughout the chromosome groups, the gaps were registered mainly in the first three groups in case of both forms of oligophrenia and also in the control.

On the base of our research we may conclude that the cells derived from the patients with Down syndrome and undifferentiated forms of olygophrenia, on the one hand, are characterized with high level of genome instability and, on the other hand, they show the different sensitivity towards the harmful effect of the mentioned compounds (BDU and metatrexat).

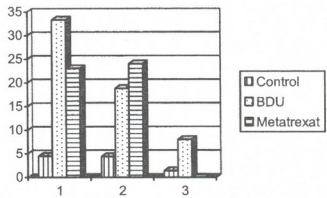

Fig. 1. The frequency of chromosome aberrations in patients with Down syndrome and undifferentiated form of olygophrenia under the influence of BDU and metatrexat. 1. Patients with Down syndrome. 2. Patients with undifferentiated form of olygophrenia. 3. Control group.

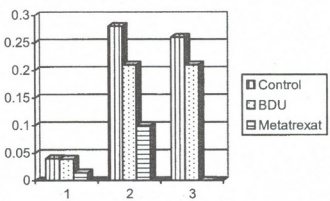

Fig. 2. The frequency of fragile sites expressed by BDU and metatrexat in patients with Down syndrome and undifferentiated form of olygophrenia. 1. Patients with Down syndrome. 2. Patients with undifferentiated form of olygophrenia. 3. Control group.

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ქრომოსომული არასტაბილურობის განსაზღვრა დიფერენცირებული და არადიფერენცირებული ოლიგოფრენიის შემთხვევაში

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(მიღებულია 02.06.2004)

რეზიუმე

შესწავლილია ქრომოსომული აპარატის ცვალებადობა – 5-ბრომდეზოქსიურიდინით და მეტატრექსატით ზემოქმედებისას დაუნის სინდრომითა და ოლიგოფრენიის არადიფერენცირებული ფორმით დაავადებულ პაციენტებში. გამოვლენილია როგორც გენომის არასტაბილურობის მაღალი ზოგადი დონე, ასევე დაუნის სინდრომით და ოლიგოფრენიის არადიფერენცირებული ფორმით დაავადებულ ინდივიდთა განსხვავებული მგრძნობელობა მაინდუცირებელი აგენტების დამაზიანებელი ზემოქმედებისადმი.

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Genetics

ANTAGONISTIC ACTIVITY IN ENDEMIC POPULATIONS OF WINE YEAST (*SACCHAROMYCES VINE*)

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Abstract

In three populations of wine yeast (Chrebalo, Mirzaani, Dedoplistskaro) antagonistic activities of strains have been studied. Three phenotype classes K, N and S were revealed in the populations. In Chrebalo and Dedoplistskaro populations only K2 system was revealed, but in Mirzaani population three systems K1, K2 and K4 were found. In Chrebalo population the rate of the strains with killer phenotype was 2,6%, in Mirzaani population - 1,4% and in Dedoplistskaro population - 0,4%. Clinar variability was revealed by the studied trait.

Key words: yeast, population, antagonistic activity, killer-system.

Introduction

In Sacharomyces one of the forms of intraspecies antagonism is elimination of sensitive strain cells by the protein-toxin Micocyn released by the killer-strain. The killer-strains are permanently infected by dsRNA of viral nature. It codes for α/β heterodimer protein-toxin that causes blocking of cell cycles and inhibition of DNA synthesis in sensitive forms [Riffer et. al., 2002]. Various killer systems have been revealed and studied in Sacheromyces. K1 and K2 forms are thoroughly studied. Since different authors classified killers using different strain collections and for the other systems no unified classification was performed [Nesterova, 1993; Shatirishvili et. al., 2001], different killer systems were revealed within the natural populations of wine yeasts. They occur in natural populations at different rates. Among the wine yeast forms, K2 plasmid-containing forms commonly occur [Naumov, 1975; Shatirishvili, Chuchulashvili, 2000].

Materials and Methods

In Sacharomyces, and in the wine yeast in particular, at the present time no characteristics are worked out for such intraspecies ranges as are the populations . The area of reproductive activity of the wine yeast vastly depends on drosophila, and therefore, it is rather small. For this reason, the wine yeast forms that are spread over villages and the surrounding areas were considered as populations. Material (wine sediment) was collected from 10 remote private cellars (so called micropopulations). Besides, the vine-yards belonging to the private farmers were not near. The samples of wine sediment were obtained from large clay vessel buried in ground. Wine fermentation in such permanent conditions continues spontaneously.

No productive strains were introduced into them. Each sample taken from each isolated strain was placed in a sterile vessel. With large microbiological loops we took the material and placed it into sterile feeling media (grape juice and tap-water in ratio 1:1) and incubated at 30^0C for 24 hours. Then the diluted material was transferred to the solid media containing the grape juice. Incubation lasted for 4 days at 30^0C. 50 strains from each population were isolated.

The antagonistic activities were determined by the standard test-strains: KJ(KJL-K1); S14 (sensitive to the K1); Oxford genetic strains; M437 (KJL-K2); the genetic line, created at the Institute of Genetics of Microorganisms, Russian Academy of Sciences; 7A-p192 (sensitive to the K2); Petergoff genetic line. Detection of antagonistic activity was performed according to the standard YEPDMB media [Zakharov et. al., 1984].

Results and Discussion

One of the forms of interstrain antagonism is the following phenomenon: the protein-toxin, released by one strain causes elimination of another sensitive strain cells. 1500 strains have been isolated from 3 natural populations - 500 strains from each one: Chrebalo (Racha, 530m above sea-level), Mirzaani (Kakheti, 756m) and Dedoplistskaro (Kakheti, 800m). The spectrum of antagonistic activity was determined by introduction of the strain from tested populations into the standard strain layers with the stroke of the loop. Proceeding from the revealed antagonistic activities the strains from different populations were divided into three phenotype classes: killers (K) producing the protein-toxins; sensitive (S) that perish when exposed to the toxins, and neutral (N) exhibiting immunity against the protein-toxin. In killer strain cells the ability to synthesize the toxin always correlates with resistance to it. Such a resistance of a cell to its own toxin does not determine its resistance to other toxins released by other strains. Every strain can be characterized by its sensitivity to killer-toxins. As regards the killer-strains, they are characterized by the spectrum of influence on other killer, or non killer- strains.

According to their antagonistic activities, strains of different populations are grouped on the base of three phenotype classes: K, N and S. All three populations, examined by us, were found to be polymorphic by the studied trait. They were divided into morphs according to their relations to test-cultures. Here we present Mirzaani population, as a sample (Table 1).

11 morphs were identified in that population. 7 strains (1,4%) revealed antagonistic activity and they were grouped in three morphs. 453 strains (90,6%) were found to have neutral phenotype, and 40 strains (8%) of the population were sensitive. K1 plasmid-containing strains caused lysis of the cell of other system (K1-K3). K2 showed the analogous spectrum. K3 strains caused lysis of K1-strain cells, but were neutral to the other strains. K4 strains slightly affected cells of K2 system, but caused intensive lysis of K1 and K3-strain cells [Nesterova, 1993]. Thus, in Mirzaani population K1, K2 and K4 systems are met. As to the Chrebalo and Dedoplistskaro populations, only K2 plasmid was revealed in the frequencies of 2,6% and 0,4%, respectively. Different rates of the strains with K, N and S phenotypes are met in micropopulations. As a pattern, the data of 10 micropopulations from Mirzaani population are represented. The strains with antagonistic activities were found only in 2 micropopulations. The neutral forms were most frequent. In 4 subpopulations no sensitive forms were seen (Table 2).

In studied populations K, N and S phenotype classes were registered in different rates (Table 3).

The content of the strains of killer phenotype decreased in parallel with the increase of sea-level. It should be noticed, that the rate of killer forms in the populations was very low compared with the endemic populations of wine yeast we had studied before. In Kvareli population the amount of killer strains had been 95,5%, in Sobis population - 58,6%, in Kahatela - 22,4% [Shatirishvili et. al., 2001].

Table 1. Determination of antagonistic activity of Mirzaani natural population

Phenotype classes	Number of classes	tests-strains			
		M_{437}	$7A\text{-}P_{192}$	K_7	S_{14}
I	1	K	N	K	N
II	5	K	N	N	N
III	1	N	N	K	N
IV	353	N	N	N	N
V	7	N	N	N	S
VI	8	N	N	S	N
VII	4	N	S	N	N
VIII	15	S	N	N	N
IX	1	N	N	S	S
X	1	S	S	N	N
XI	2	S	N	S	N

Table 2. Determination of the frequencies of K, N, and S phenotypes in Mirzaani micropopulations

Micropopulation	Number of analyzed strains	K Killer		N Neutral		S Sensitive	
		Number	%	Number	%	Number	%
I	50	-	-	43	86	7	14
II	50	1	2	42	84	7	14
III	50	-	-	50	100	-	-
IV	50	6	12	44	88	-	-
V	50	-	-	45	90	5	10
VI	50	-	-	49	98	1	2
VII	50	-	-	38	76	12	24
VIII	50	-	-	42	84	8	16
IX	50	-	-	50	100	-	-
X	50	-	-	50	100	-	-
Total	500	7	1,4	453	90,6	40	8

Table 3. Frequency of K, N and S phenotype classes in wine yeast natural population

Population	Number of studied strains	Number of morphs	K		N		S	
			Number	%	Number	%	Number	%
Chrebalo "Aleksandrouli"	500	18	13	2,6	382	76,4	105	21
Mirzaani "Rkatsieli"	500	11	7	1,4	453	90,6	40	8
Dedoplistskaro "Kaberne"	500	12	2	0,4	445	89	53	10,6

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ანტაგონისტური აქტივობა ღვინის საფუარის (*Saccharomyces vine*) ენდემურ პოპულაციებში

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რეზიუმე

შესწავლილია ღვინის საფუარის 3 პოპულაციაში (ჭრებალო, მირზაანი, დედოფლის-წყარო) ანტაგონისტური აქტივობა. პოპულაციებში გამოვლე-ნილია 3 ფენოტიპური K, N და S კლასი. ჭრებალოსა და დედოფლისწყაროს პოპულაციებში გვხვდება მხოლოდ K2 სისტემა, ხოლო მირზაანის პოპულა-ციაში - K1, K2 და K4 სისტემები. ქილერი ფენოტიპის მქონე შტამების სიხშირე ჭრებალობის პოპულაციაში 2,5%-ია, მირზაანში - 1,4%, ხოლო დედოფლისწყაროში - 0,4%. გამოვლენილია ანტაგონისტური აქტივობის მიხედვით კლინარული ცვალებადობა.

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Genetics

THE ANTIMUTAGENIC EFFECT OF THE SYNTHETIC PEPTIDE PROSTAMAX IN HUMAN

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Abstract

The effect of synthetic biopeptide - Prostamax at therapeutic concentration (0,01μg/ml) has been studied on the Mercury-chloride induced short-term lymphocyte cultures of 20-30 year clinicaly healthy individuals. The antimutagenic effect of tested peptide has been shown.

Key words: Prostamax, antimutagen, Mercury-chloride, chromosome, aberration, aneuploidy.

Introduction

A special interest is drawn to a new class of pharmaceuticals - peptide bioregulators, that regulate tissue-specific processes, increase secretion of regulatory messengers, change the genome functional activity and regulate protein syntheses and the processes of cell proliferation and differentiation due to impact on the m-RNA transcription and the gene expression. They activate the DNA-reparation, which represents the bases of cellular homeostasis [Khavinson et al. 2003., Shataeva et al. 2003., Lezhava et al. 2003].

Mercury salts represent the compounds with stated mutagenic activity in many species, especially in man. It is known that they induce both gene and chromosome mutations and reveal a dose-dependent effect [Ben-Ozer et al.2000., Buccio et al.1999., Jokhadze et al. 2003., Shocny R. 1996].

Thus, antimutagenic and protective effect investigation of regulatory agents is of interest. The aim of present research was to study the effect of synthetic peptide Prostamax at therapeutic concentration on the Mercury-chloride treated cultured cells of human peripheral blood.

Material and methods

Investigations have been carried out on human peripheral blood cultures obtained from 5 clinically healthy persons at the age of 20-30. Lymphocytes were cultivated by the standard method. Mercury-chloride solutions at concentrations 10^{-3}M and 10^{-4} M were added to the cultures on the 24th hour and left for the entire period of incubation (72 hrs.). In the next experiment Mercury-chloride solutions at above mentioned concentrations were added to the cultures on the 24th hour and then Prostamax solution at therapeutic concentration (0,01μg/ml) was added to the cultures on the 48th hour and left for the entire period of incubation (72 hrs.).

The structural and numerical chromosome parameters were registered on the chromosome preparations. For each individual the results were compared with the own control value.

Results and discussion

In total 2500 metaphases were analysed to establish structural and numerical chromosome abnormalities induced by solutions with concentrations $10^{-}3M$ and $10^{-4}M$ of Mercury-chloride and for studying the effect of biopeptide Prostamax on the Mercury-chloride treated cultivated cells of human peripheral blood. 100 metaphases were analysed for each variant of experiment.

Significantly increased frequency of the structural and numerical chromosome abnormalities at above-mentioned concentrations of Mercury-chloride solutions was detected in all individuals (Table 1). Common chromosome damages were single and pared fragments. The total frequency of cells with chromosome aberrations at concentration $10^{-4}M$ was 14%. The total frequency of aberrant cells reached maximum value at the concentration $10^{-3}M$ was 20%. Significant individual variability according to these parameters was not detected.

Table 1. The impact of biopeptide Prostamax on the level of $HgCl_2$ induced chromosome aberration and aneuploidy

do-nors	Control		$HgCl_2$ - $10^{-4}M$		$HgCl_2$ - $10^{-3}M$		$HgCl_2$ - $10^{-4}M$ with Prostamax		$HgCl_2$ - $10^{-3}M$ with Prostamax	
	cells with chrom. aberr. %± m	cells with aneuploidy %± m	cells with chrom. aberr. %± m	cells with aneuploidy %± m	cells with chrom. aberr. %± m	cells with aneupl-oidy %± m	cells with chrom. aberr. %± m	cells with aneuploidy %± m	cells with chrom. aberr. %± m	cells with aneupl-oidy %± m
1	2,0 ± 1,4	7,0 ± 2,6	14,0 ± 3,7	13,0 ± 3,6	20,0 ± 4,4	19,0 ± 4,3	2,0 ± 1,4	10,0 ± 3,1	6,0 ± 2,4	13,0 ± 3,6
2	3,0 ± 1,7	7,0 ± 2,6	15,0 ± 3,8	16,0 ± 4,0	20,0 ± ,44	20,0 ± 4,4	3,0 ± 1,7	11,0 ± 3,3	5,0 ± 2,2	15,0 ± 3,8
3	2,0 ± 1,4	7,0 ± 2,6	14,0 ± 3,7	11,0 ± 3,3	18,0 ± 4,2	18,0 ± 4,2	4,0 ± 2,0	9,0 ± 3,0	7,0 ± 2,6	14,0 ± 3,7
4	3,0 ± 1,7	5,0 ± 2,2	12,0 ± 3,4	11,0 ± 3,3	23,0 ± 4,7	18,0 ± 4,2	3,0 ± 1,7	11,0 ± 3,3	6,0 ± 2,4	13,0 ± 3,6
5	3,0 ± 1,7	7,0 ± 2,6	15,0 ± 3,8	12 ± 3,4	19,0 ± 4,3	18,0 ± 4,2	4,0 ± 2,0	10,0 ± 3,1	7,0 ± 2,6	14,0 ± 3,7
total	2,6 ± 1,6	6,6 ± 2,5	14,0 ± 3,7	12,6 ± 3,5	20,0 ± 4,4	18,6 ± 4,3	3,2 ± 1,7	10,2 ± 3,1	6,2 ± 2,4	13,8 ± 3,7

Frequency of aneuploidy also was increased. Total frequency of cells with aneuploidy was 12,6% at concentration $10^{-4}M$ and 18,6% at concentration $10^{-3}M$. Thus, there were detected significant mutagenic effects of Mercury-chloride solutions at concentration $10^{-4}M$ and $10^{-3}M$.

1000 metaphases were analysed for studying the effect of synthetic peptide Prostamax on the Mercury-chloride added cultivated cells of human peripheral blood. Significant antimutagenic effect of Prostamax at therapeutic concentration (0,01μg/ml) was detected (Table 1). Total frequency of cells with chromosome aberrations was 3,2% in combination with $10^{-4}M$ $HgCl_2$ and 6,2% in combination with $10^{-3}M$ $HgCl_2$. These results reliably differ from the above described data. Total frequency of cells with aneuploidy was 10,2% in combination with $10^{-4}M$ $HgCl_2$ and 13,8% in combination with $10^{-3}M$ $HgCl_2$.

Thus, there was detected antimutagenic effect of synthetic biopeptide Prostamax in the Mercury-chloride treated lymphocyte cultures of human peripheral blood. We suppose that

Prostamax activates the DNA-reparation processes due to chromatin reactivation through its ability to modify heterochromatinized chromosome regions, that makes the DNA molecule accessible for reparation systems. These results are in agreement with the data indicating the chromatin activating ability of synthetic bioregulator peptide [Khavinson et al.2003., Lezhava et al. 2003].

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სინთეზური ბიორეგულატორული პეპტიდის პროსტამაქსის ანტიმუტაგენური მოქმედება ადამიანში

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რეზიუმე

შესწავლილია სინთეზური ბიორეგულატორული პეპტიდის – პროსტამაქსის თერაპიული კონცენტრაციის (0,01μg/ml) გავლენა ვერცხლისწყლის ქლორიდის მუტაგენური კონცენტრაციებით (10^{-4} M და 10^{-3} M) ინდუცირებული 20-30 წლის კლინიკურად ჯანმრთელ ინდივიდთა სისხლის ლიმფოციტების მოკლევადიან კულტურებზე. ნაჩვენებია აღნიშნული პრეპარატის ანტიმუტაგენური ეფექტი.

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Genetics

DETERMINATION OF THERMOSENSITIVE PERIOD IN CONDITIONALLY LETHAL RADIOSENSITIVE MUTANTS

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Abstract

In UV-rays induced 67 conditionally lethal radiosensitive mutants with damaged reparation, blocked mitosis and meiosis at 37°C temperature, period of thermosensitivity has been identified. Mutants were associated into 10 morphs. Mutants with disordered DNA synthesis and meiosis are associated into 1-4 morphs, while the rest morphs contain mutants with disordered ascogenesis.

Key words: yeast, UV-rays, sensitive mutation, meiosis.

Introduction

Differences in radiosensitivity of various species of microorganisms as well as various strains of certain species seems to be one of the most disputable issues of modern radiobiology. Resistance of microorganisms towards irradiation is a complex feature and is determined by cell genotype [Fousteri, Lehman, 2000].

Reparation of genetic material greatly depends on the replication and recombination processes, that acquire general stages and are led by similar enzymes. Mutations in the genes responsible for replication, reparation and recombination processes lead to pleiotropic effects. Several genes rad, cdc and spo controlling certain stages of reparation, mitosis and meiosis have been revealed [Henninger-Rutkovski, Esposito, 2000; Esposito, et al., 2001; Nurse et al., 1998]. Disordered meiosis, high temperature blocked mitosis of UV-rays induced conditionally lethal radiosensitive mutants of wine yeasts have been studied [Zarnadze, 2003].

Materials and Methods

Experiments were carried out on UV-rays induced conditionally lethal radiosensitive mutants of *Sacharomices cerevisae var. vini* strain CU 90. Complete pepton-containing medium and acetate medium for inducing sporulation has been used. Culture medium was described previously [Zakharov et al., 1980]. In order to define mutants' disordered thermosensitive duration, yeast cell culture was transferred from high temperature (37° C) to optimal (30° C) and visa versa during meiosis and sporulation with 6 hour intervals within two days. Percentage of ascs in culture was calculated by Goriaev Chamber.

Results and Discussion

To reveal conditionally lethal radiosensitive mutants' imperfect function during sporulation cycle, two series of experiments at alternative temperatures have been carried out. Inducing sporulation of tvs mutants at optimal temperature cells were transferred after different intervals to high temperature conditions, resulting in the repression of mutants gene expression. Thus, the period of time after which mutants don't need the optimal temperature for gene expression has been identified.

Data obtained revealed ascs percentage alterations of mutant strains when the temperature regime was changed. In particular, incubation of sporulation was performed at 37°C by subsequent transfer of cells to optimal temperature conditions (30°C). Increase of incubation temperature up to 37°C results in gradual irreversible blocking of ascs formation.

Fig.1 illustrates alterations of ascs formation observed during transfer of three mutant cells - tvs 33, tvs 65 and tvs 69. Considering ascs formation value as critical point equal to O%, it appears that cell transfer from 30°C up to 37°C represents the margin of sensitivity period, i.e. the point where mutants reveal sensitivity to enhanced temperature. Downward curve of cell transfer data from 37°C to 30°C indicates the exact point, when mutants gene expression is turned off leading to irreversible blocking of sporulation.

Investigation of conditionally lethal radiosensitive tvs mutants incubated at alternative temperature conditions may have implications in understanding of sensitive stage controlled by the mutated gene during sporulation. Data obtained enable us to divide mutants into 10 morphs (Fig.2).

TU 90 strains' ascogenesis has been studied. Comparing asc formation process and DNA synthesis [Diffey, 1995; Kyntrel et. al 1996] during meiosis to data obtained for tvs mutants, the following conclusion should be made: in 1-3 morphs mutants the process of DNA synthesis and meiosis are disordered, while 4-10 morphs mutants represent disturbance of different stages of ascogenesis.

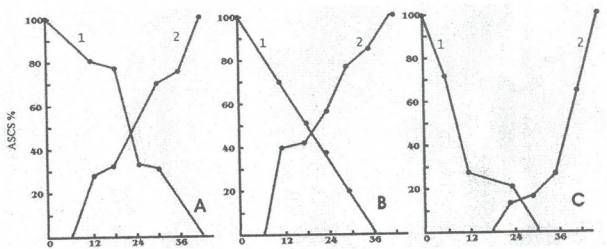

Fig.1 Ascogenesis of tvs mutants developed at different temperature:
A – mutant tvs 33; B - mutant tvs 65; C - mutant tvs 69; 1 - Mutants transfer from 37°C to 30°C; 2 - Mutants transfer from 30°C up to 37°C (x axis - incubation time, y axis - asc formation in %).

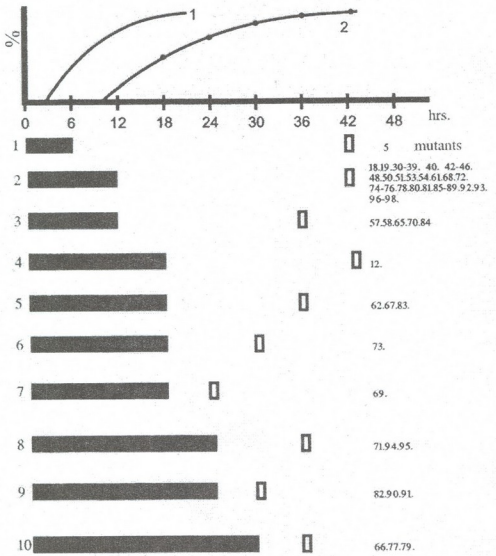

Fig. 2. Identification of thermosensitive period of tvs mutants

1 – DNA sunthesis [Esposito et al., 1969; Kuntzell et al., 1996]; 2 – Ascogenesis of CU 90 strain-control. ■ - the period of time when the mutants express sensitivity to high temperature. □ – expression of blocking in mutants resulting in irreversible halting of sporulation.

Functional test on tvs mutants alleles has been developed. Test cultures were cdc 28 and rad 9 mutants. Assessed tvs mutants' loci appeared not to be allelic to cdc 28 and rad 9 mutations; tvs mutations were distributed into 12 different loci [Zarnadze et. al 2003].

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თერმომგრძნობელობის პერიოდის განსაზღვრა პირობითლეტალ რადიომგრძნობიარე მუტანტებში

ზარნაძე თ., ჯორბენაძე ც., კაჭარავა მ., შათირიშვილი ა.

გენეტიკის კათედრა, უჯრედული და მოლეკულური ბიოლოგიის კათედრა, ივ. ჯავახიშვილის სახელობის უნივერსიტეტი

(მიღებულია 03.05.2004)

რეზიუმე

ულტრაიისფერი სხივებით ინდუცირებულ 67 პირობითლეტალურ მუტანტში, რომელშიც დარღვეულია რეპარაციის პროცესი ხოლო 37^0C ტემპერატურაზე ბლოკირებულია მიტოზი და მეიოზი, კარტირებულია თერმომგრძნობელობის პერიოდი. მუტანტები გაერთიანებულია 10 მორფაში. 1-4 მორფაში გაერთიანებულ მუტანტებში დარღვეულია დნმ-ის სინთეზი და მეიოზი, დანარჩენ მორფაში გაერთიანებულ მუტანტებში კი ასკოგენეზი.

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Microbiology

MICROSCOPIC FUNGI FROM VARIOUS REGIONS OF GEORGIA

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Abstract

186 pure cultures of microscopic fungi from different soil-climatic zones of Georgia (subalpine, subtropical forest-steppe, dry subtropical steppe) have been isolated and cultivated as pure cultures. Identification of the isolated cultures has shown that soil microflora of the mentioned climatic zones is presented by micromycetes belonging to the classess *Zygomycetes, Ascomycetes* and *Deuteromycetes*. The following genera were distinguished by the highest incidence: in subalpine zone - the genera *Fusarium* and *Mucor*; in the zone of subtropical forest-steppe - the genera *Aspergillus* and *Penicillium*; in the zone of dry subtropical steppe - the genera *Aspergillus* and *Trichoderma*. Different combination of micromycetes' genera is characteristic not only for the group of soils of the definite zone, but for each concrete soil type itself.

Key words: microscopic fungi, soils, genus, *Zygomycetes, Ascomycetes, Deuteromycetes*.

Introduction

Investigation of morphogenetic and biochemical peculiarities of fungi able for growth in extremal and sub-extremal conditions is important trend of modern mycology [Hakamada Y. et al, 1997]. Many species of soil microscopic fungi are known to be active producers of different enzymes (micromycetes of *Aspergillus, Penicillium, Trichothecium* and some other genera are the producers of cellulases; the species of the genera *Aspergillus, Chaetomium* and *Fusarium* – the producers of xylanases; *Aspergillus* and *Fusarium* – the producers of pectinases; micromycetes of *Mucor*, *Rhizopus* and some other genera - producers of proteases) [Bilai V., 1989]. Therefore, extremophilic microscopic fungi are of special interest as they are able to produce the enzymes resistant to different critical conditions [Kvesitadze G., 1990]. At the same time some representatives of microscopic fungi possess ability of assimilation and degradation of organic toxicants [Bayman P., Radkar G., 1997].

Soil microflora is extremely rich and diverse by composition. Complex interrelations between biotic, physicochemical and geographical factors are responsible for the formation of certain ecological groups of fungi comprised of species adapted to definite natural conditions [Bilai V., 1989].

The present investigation was aimed at isolation of microscopic fungi from soils of different regions of Georgia, obtaining of pure cultures of isolated fungi and identification of microscopic fungi from the created collection to select the micromycetes able for growth in extreme conditions.

Materials and Methods

Microscopic fungi were isolated from the averaged soil sample obtained by mixing of several samples. Soil samples were taken by the generally applied technique. Sowing of samples was performed during the first day-night from sample taking. The samples were dispersed according to the method by Zvyagintsev [Zvyagintsev D., 1980].

Fungi were isolated from the soil according to the Waksman's method of soil dilution. Sowing of micromycetes was performed in the depth of agarized nutrition medium on Petri dishes. The dishes were incubated in the thermostat at 28°C temperature for 10-15 days and were monitored every 2 day-nights starting from the 3rd day.

Nutrition media were chosen according to the principle of selectivity. Microscopic fungi able to easily uptake accessible carbohydrates were isolated using the agarized beer molasses and Czapeck's medium. In order to isolate cellulose-degrading micromycetes sowing was performed on the modified Czapek-Doks medium with microcrystalline cellulose as the only carbon source [Segi I., 1983] and on the Hatchinson and Cleyton's medium. In the latter case sowing was made according to the method by Pushkinskaya [Zvyagintsev D., 1980].

To avoid propagation of bacteria pH of the media was 4.0-4.2. As such level of acidity in the medium is limiting for growth of some species of fungi, sowing was performed on the medium with beer molasses (pH 6.0) with added antibiotics.

The isolated microscopic fungi were identified using the methods of microscopy, namely the technique of pressed drop and the colony prints preparation [Segi I., 1983]. The preparations were made in the mixture of alcohol with glycerin and water at the ratio 1:1:1. Fixation of the preparation was made both thermally and chemically with 96° ethyl alcohol of Nikiforov's solution [Segi I., 1983]. The preparations were stained with alcohol solution of methylene blue.

Results and Discussion

It has been revealed that each soil type and plant association is characterized by specific composition of micromycetes. Species composition of soil micromycetes is essentially influenced by such external factors as temperature, pH of the medium, humidity. Physicochemical and mechanical features of the soil affect the formation of soil mycoflora allowing to reveal extreme conditions for growth and propagation of certain species of fungi. To create the collection of micromycetes adapted to extreme conditions, soil samples were taken from different soil-climatic zones of Georgia. In each region different soil types were chosen. In particular, Oni region (subalpine zone), characterized with humid climate, cold winter and chilly short-term summer (soddy mountain-meadow, humic-carbonate and podzolized brown soils); Telavi region (zone of subtropical forest-steppe) with moderately humid climate, cold winter and hot summer (alluvial acid, solonetz alkali-soil and chernozem soils); Sighnaghi region (zone of dry subtropical steppe) with humidity coefficient <1 (meadow chernozem, brown carbonate and chestnut soils [Atlas of Soils of Georgia, 1984].

From the above mentioned soils 186 pure cultures of microscopic fungi have been isolated (Fig. 1). The obtained cultures were identified using different methods of microscopy and guides [Malloch D., 1981]. Class, order, family and genus of isolated fungi have been determined. For each investigated soil type the incidence of individual genera and dominating ones have been determined.

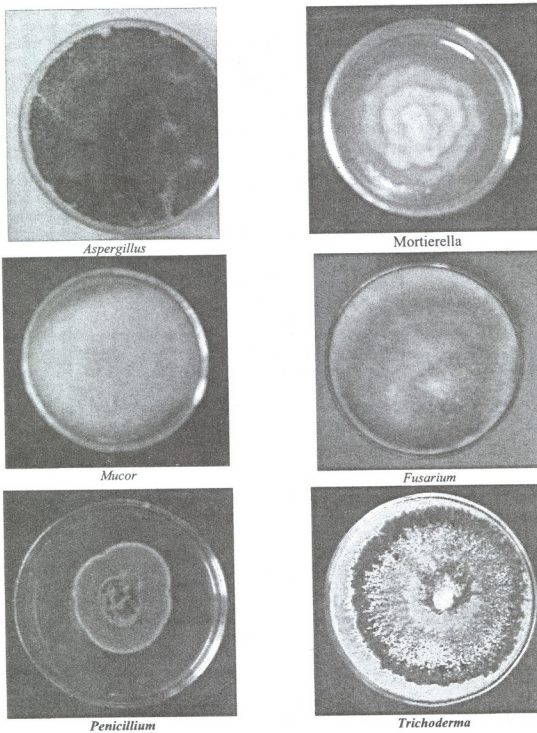

Fig. 1. Colonies of microscopic fungi isolated from different soils of Georgia.

Identification of the isolated microscopic fungi revealed, that microflora of all three climatic zones is presented by different proportions of microscopic fungi belonging to the classes *Zygomycetes, Ascomycetes* and *Deuteromycetes*. The soils of various zones as well as different soil types of one and the same zone had distinct generic composition (Table 1). For example

Chernozem (black earth) soil from Telavi region was distinguished by special diversity of genera belonging to the classes *Ascomycetes* and *Deuteromycetes*. Both the genera characteristic to all soil types and the rare or absent ones in other types of soil (for example: *Petromyces, Sporotrichum, Botrytis, Trichothecium)* have been isolated from the soil. From above mentioned classes only the genera *Aspergillus, Penicillium* and *Trichoderma* were found in the saline solonetz soil of the same zone. This may be explained by the fact, that chernozem soils contain sufficient amounts of nitrogen, potassium and phosphorus and pH approaches 6.0, while the saline solonetz soils are alkaline (pH=8.4).

Table 1. Generic composition of microscopic fungi isolated from soils of Telavi, Sighnaghi and Oni regions. I -alluvial acid soil; II - solonetz alkali-soil; III – chernozem soil; IV – brown carbonate soil; V – chestnut soil ; VI – black meadow-soil; VII – sod mountain-meadow soil; VIII – humic carbonate soil; IX – brown podzolized soil.

Genus of the fungus		Telavi			Sighnaghi			Oni		
		I	II	III	IV	V	VI	VII	VIII	IX
Zygomycetes	*Absidia*								+	+
	Mortierella						+		+	+
	Mucor	+	+	+	+		+	+	+	+
	Rhizopus	+				+	+	+	+	+
Ascomycetes	*Aspergillus*	+	+	+	+		+		+	
	Penicillium	+	+	+	+	+	+		+	+
	Petromyces			+						
	Chaetomium				+	+	+			
Deuteromycetes	*Botrytis*				+		+			
	Cladosporium	+		+			+		+	
	Fusarium	+		+	+	+	+	+	+	+
	Trichoderma		+		+	+	+			
	Trichothecium			+						
	Sporotrichum			+						

In Oni region humic-carbonate soil was distinguished from other soil types by the variety of microscopic fungi (Table 1). This fact is in agreement with consideration that soils with rich vegetation cover are characterized with great specific diversity of micromycetes [Bilai V., 1989]. Soil microflora of subalpine zone of Oni region is distinct from those of Sighnaghi and Telavi districts. It is mainly represented by the genera of Mucorales family, which is characteristic to the zones with humid climate. It should be mentioned, that though according to the literature the genera *Trichoderma* and *Mortierella* are the most spread typical soil fungi, in our experiments *Trichoderma* was isolated only from the soils of subtropical zone and *Mortierella* only from the soils of subalpine zone.

The dominating frequently occurring genera, which usually determine the type of micromycetes association, were: for subalpine zone of Oni region - *Fusarium* and *Mucor*; for Telavi zone - *Aspergillus* and *Penicillium*; for the zone of Sighnaghi *Aspergillus* and *Trichoderma* (Fig. 2). In all investigated soils the number of rarely occurring genera exceeded that of frequently

occurring ones. In Telavi zone the genera *Mucor, Rhizopus, Petromyces, Sporotrichum, Cladosporium* and *Trichothecium* were rare; in Sighnaghi zone - the genera *Mucor, Chaetomium, Penicillium, Botrytis, Trichoderma, Fusarium* and *Rhizopus*; In Oni region - *Penicillium, Absidia, Mortierella, Aspergillus.*

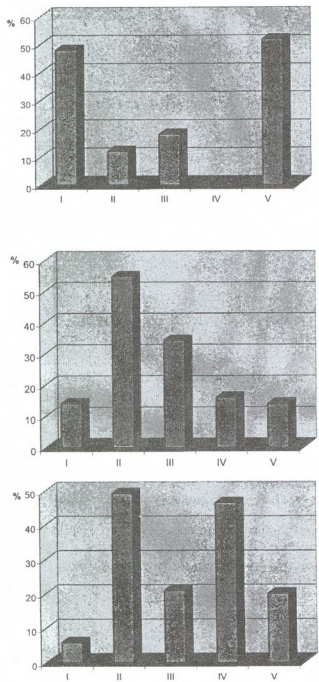

Fig. 2 Incidence of some genera of microscopic fungi in different soil-climatic zones of Georgia. I – *Mucor*, II – *Aspergillus*, III – *Penicillium*, IV – *Trichoderma*, V - *Fusarium* .

Thus, the comparison of the obtained results shows, that soil microflora of different climatic zones of Georgia sharply differs from each other by generic composition and in the whole is distinguished by the great diversity. Different combination of micromycetes' genera is characteristic not only for the group of soils of the definite zone, but for each concrete soil type itself.

Acknowledgement: This work was supported by STCU Project #G-101.

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საქართველოს ზოგიერთი რეგიონის ნიადაგებიდან მიკროსკოპული სოკოების გამოყოფა

დაუშვილი ლ., ჭუთათელაძე ლ., ბურდული თ., ჯობავა მ., ძალამიძე ი. ალექსიძე თ., თინიკაშვილი ლ.

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

(მიღებულია 17.11.2003)

რეზიუმე

საქართველოს სხვადასხვა კლიმატურ-ნიადაგური ზონიდან (სუბალპური, სუბტროპიკული ტყე-სტეპის, მშრალი სუბტროპიკული სტეპის) გამოყოფილი და სუფთა კულტურების სახით მიღებულია 186 მიკროსკოპული სოკოს კულტურა. მათი იდენტიფიცირებით დადგენილია, რომ აღნიშნული კლიმატური ზონების ნიადაგური მიკროფლორა წარმოდგენილია *Zygomycetes, Ascomycetes* და *Deuteromycetes* კლასების მიკრომიცეტებით. არსებობის მაღალი სიხშირით გამოირჩეოდნენ სუბალპურ ზონაში გვარები *Fusarium* და *Mucor*, სუბტროპიკულ ტყე-სტეპის ზონაში – *Aspergillus* და *Penicillium*, სტეპის ზონაში – *Aspergillus* და *Trichoderma*. არა მარტო განსაზღვრული ზონის ნიადაგების ჯგუფისათვის, არამედ ყოველი კონკრეტული ნიადაგისათვის დამახასიათებელია მიკრომიცეტების გვარების ერთმანეთისაგან განსხვავებული შეხამება.

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Microbiology

ISOLATION AND IDENTIFICATION OF MICROSCOPIC FUNGI FROM SOME SOIL - CLIMATIC ZONES OF THE CAUCASUS

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Abstract

68 microscopic fungi have been released from different soil and climatic zones of Georgia (sub-alpine Kazbegi region and humid-subtropical Poti region), and identified till the genus. The dominant genera characteristic for the soils of these regions and the frequency of their existence was determined. The regularity of diffusion of different microscopic fungi stipulated by the ecological and geographical conditions and fungal physiology was studied. Genus *Penicillium* appeared to be dominant for both soil-climatic zones. Genus *Fusarium* was characteristic for Poti region soils and was absent in Kazbegi region soils, while genus *Absidia* was found only in sub-alpine soils.

Key words: pure culture, dominant, *Aspergillus, Penicillium*

Introduction

The microbiological synthesis became intensively increasing direction among the different industrial branches during last 20 years. On the base of technologies of stable enzymes in the nearest future it will become possible to solve problems like ecologically pure products obtaining, progress of energetically economical technologies, intensive insertion of bioremediation in conservancy, creation of new multifold waste less technologies [Adams M., 1993; Bhat M., 1997].

For the elaboration of new technologies of microbiological changes, it becomes necessary to search a new species of microorganisms. For this point of view the microscopic fungi are of great interest. As eukaryotes, they posses genetically wider information compared with prokaryotes, and are able to perform different microbiological transformations.

Microflora of the Caucasus, comprising more than 20 different soil-climatic zones is one of the interesting regions for studing microscopic fungi. The goal of our work is to make up the collection of mycelial fungi of different ecological niches of the Caucasus.

Materials and Methods

10 g of soil samples were picked in two different ecological niches of Caucasus: subalpes (Kazbegi region - mountain-alley soils) and humid subtropics (Poti region - marshy and podzol soils) [Fomin et al., 2001].

To obtain the homogenous suspension containing separately and freely moving cells of microorganisms, the samples were previously treated using the method of soil aggregates

dispersing [Zvyagintsev, 1980]. Treated material was sowed on a sterile Petri dishes by Waksman's method of soil dilution and Warcup's direct sowing method. For this purpose suspensions with following dilution were prepared: 10^1, 10^2, 10^3 and 10^4.

The microscopic fungi were isolated on the following nutrient mediums: (g/l) 1) The universal medium - 0.51 7°B wort, 0.5l tap water, 20g agar, pH – 5.5-6.0. 2) Chapek's acidified medium (for inhibition of bacteria) – $NaNO_3$-9.1, KH_2PO_4- .0, $MgSO_4$-0.5, KCl-0.5, $FeSO_4$ - 0.02, glucose-40.0, agar- 0.0, pH - 3.5–4.2. 3) Selective nutrient medium – $NaNO_3$-3.2, KH_2PO_4-2.0, $MgSO_4$-0.5, yeast extract-10.0, microcrystal cellulose-1%, agar-20.0, pH - 5.5–6.0. 4) Chapek's modified medium – $NaNO_3$-9.1, KH_2PO_4-1.0, $MgSO_4$-0.5, KCl-0.5, $FeSO_4$-0.02, starch-20.0, agar-20.0, pH - 5.5–6.0. 5) Chapek-Dox's nutrient medium – $NaNO_3$-2.0, KH_2PO_4-1.0, $MgSO_4 \cdot 7H_2O$-0.5, KCl-0.5, $FeSO_4 \cdot H_2O$-0.02, sucrose-30.0, agar-20.0. Nutrient mediums were sterilized under 0.6 atm for 45 min, in autoclaves.

Incubation of the microscopic fungi obtained from the soil samples was performed at 30°C in thermostat. Plate cultures were observed on the 3rd, 5th, 7th and 10th days. On the 10th day the separate colonies of cultures were sowed once more and cultivated at 30°C for 10 days. The piece of mycelium or a small portion of spores from plate culture were placed in the test-tube containing sterilized universal medium. Test-tubes were placed into the thermostat at the same temperature for 10 days.

The frequency of detection of particular species was determined by the ratio: $FD = \frac{IS}{TS}$

(FD – Frequency of species detection, IS – amount of the investigated sample, TS – Total amount of the sample).

Morphological and cultural peculiarities of the cultures were studied by light microscopy. Also different guides were used [Bilaiy V., 1988, Malloch D., 1981]. In some cases the dry optical system microscopy was used.

Results and discussion

35 different species of microscopic fungi has been distinguished from the soils of geographically distant territories of the subtropical zones. 33 ones were released from the soils of sub- alpine zone (at 3000m, 3800m, and 4200m above sea level).

While using dilution method, 10^2 and 10^3 dilutions turned to be less optimal. Small dilution (10^1) caused over-covering of the colonies. In the case of high dilution all colonies "disappeared".

Micromycetes cultures were obtained on the different nutrient mediums. Universal nutrient medium distinguished with the abundant microflora with 52.86% isolation of microscopic fungi (Table 1). Less diverse was the microflora of Chapek's acidified and modified nutrient mediums. The selective and Chapek-Doks mediums also turned to be very poor with microscopic fungi.

According to the experimental results the difference was revealed not only between the microflora of diverse climatic-soil zones, but also among the soils of one and the same geographically distant climatic zones (Tables 2, 3).

In Poti region soils genera *Fusarium* and *Penicillium* were prevailed (Table 2). Maltakva region was distinguished by its microfloral diversity, all genera characteristic for the humid-subtropical soils were spread here (except genera: *Chaetomium, Rizopus, Botritis*)

Monotonic was the microflora of Grigoleti, where the genus *Fusarium* absolutely predominated. The representatives of the genus *Botritis* were found only in Grigoleti, while genera *Chaetomium* and *Rizopus* present only in the soils of Poti coast pine forests (Table 2).

Table 1. Releasing of microscopic fungi on different nutrient mediums

Nutrient medium	Quantity of released micromycetes (%)	
	Poti region	Kazbegi region
Universal	52.86	51.62
Chapek's	25.71	24.24
Chapek's modified	12.86	15.15
Selective	5.71	6.03
Capek-Doks's	2.86	3.03

Table 2. Microflora of Caucasian humid-subtropical zone (Poti region)

Genera	Frequency of detection, %		
	Maltakva	Grigoleti	Coastal pine forest in Poti
Fusarium	29.42	66.67	33.33
Trichoderma	23.53	-	-
Penicillium	17.65	-	26.67
Aspergillus	11.76	-	-
Allescheria	5.88	-	-
Mucor	5.88	-	-
Mortierella	5.88	-	13.33
Botritis	-	33.33	-
Chaetomium	-	-	6.67
Rizopus	-	-	20.00

Identification of Kazbegi region soils revealed that the dominants of subalpine zone are representatives of genera: *Mucor, Mortierella* and *Penicillium* (Table 3). The last one wasn't discovered on the 4200m. The influence of ecological and geographical factors on the distribution of different genera is especially clear in subalpine zone. Here representatives of particular genus replace each other: genus *Allescheria* is spread only at 3000m, representatives of Deoteromycetes (genus *Cladosporium*) dominate at 3800m, while genus *Absidia* turned to be the only at the 4200m.

Comparing the microflora of humid and subalpine soil-climatic zones (Table 4) revealed the diversity of Poti region soils' microflora constitution. As the "property" of subalps, genera *Absidia* and *Cladosporium* don't occur here.

Table 3. Microflora of Caucasian subalpine soil-climatic zone (Kazbegi region)

Genera	Frequency of detection, %		
	3000m above s.l.	3800m above s.l.	4200m above s.l.
Penicillium	35.71	30.0	-
Absidia	-	-	55.56
Mucor	21.43	20.0	33.33
Mortierella	21.43	30.0	11.11
Aspergillus	14.29	10.0	-
Allescheria	7.14	-	-
Chaetomium	-	10.0	-

Table 4. Comparing the microflora of two Caucasian soil-climatic zones (humid subtropical and subalpine)

Genera of Micromycetes	Frequency of detection, %	
	Poti region	Kazbegi region
Fusarium	34.28	-
Penicillium	20.0	24.24
Trichoderma	11.43	-
Mortierella	8.57	21.21
Rizopus	8.57	-
Aspergillus	5.71	9.09
Botritis	2.86	-
Chaetomium	2.86	-
Mucor	2.86	24.24
Allescheria	2.86	3.03
Absidia	-	15.15
Cladosporium	-	3.03

Summarizing we can say that identified fungi belonged mainly to Zygomycetes (genera: *Absidia, Mucor, Martierella, Rizopus*), Ascomycetes (genera: *Aspergillus, Penicillium* and *Chaetomium*) and Deiteromycetes (genera: *Cladosporium, Fusarium, Trichoderma, Botritis* and *Allescheria*) classes.

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კავკასიის ზოგიერთი ნიადაგობრივ-კლიმატური ზონიდან მიკროსკოპული სოკოების გამოყოფა და იდენტიფიცირება

ჭუთათელაძე ლ., იაშვილი თ. , ზაქარიაშვილი ნ., ალექსიძე თ., საბაშვილი ნ., აჰლაკოვ ვ., ხოხაშვილი ი., ჯობავა მ.

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

(მიღებულია 10.11. 2003)

რეზიუმე

კავკასიის ორი განსხვავებული ნიადაგურ-კლიმატური ზონის, კერძოდ სუბალპური (ყაზბეგის რაიონი) და ტენიან-სუბტროპიკული (ფოთის რაიონი), ნიადაგებიდან გამოყოფილია და გვარამდე იდენტიფიცირებულია 68 მიკროსკოპული სოკო. დადგენილია ამ რეგიონის ნიადაგებისათვის დამახასიათებელი დომინანტური გვარები და განსაზღვრულია მათი შეხვედრის სიხშირე. დადგენილია მიკროსკოპული სოკოების სხვადასხვა გვარების გავრცელების კანონზომიერება. ორივე ნიადაგობრივ-კლიმატური ზონისთვის დომინანტურ გვარს წარმოადგენს *Penicillium*-ი. ფოთის რეგიონის ნიადაგების პირველი რანგის დომინანტია *Fuzarium*-ის გვარი, რომელიც საერთოდ არაა გავრცელებული ყაზბეგის რაიონის ნიადაგებში. ამავე დროს, გვარი *Absidia* მხოლოდ სუბალპური ზონიდან იქნა გამოყოფილი.

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Molecular Biology

MODULATION OF DNA-TOPOISOMERASE II ACTIVITY BY ADP-RIBOSYLATION IN THE NUCLEAR MATRIX OF EUKARYOTIC CELLS

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Abstract

Using DNA electrophoresis in the pulse field it has been shown that ADP-ribosylation in the nucleoids of human mononuclear leukocytes and rat brain cortex neurons stimulates the DNA loops cleavage at the DNA attachment sites to the nuclear matrix. Possible participation of ADP-ribosylation in the modulation of the activity of DNA-topoisomerase II in the nuclear matrix of eukaryotic cells was suggested.

Key words: DNA, NAD, nuclear matrix, poly(ADP-Ribose)polymerase, topoisomerase II, leukocytes, neurons.

Introduction

ADP-ribosylation is a reversible, covalent, post-translational modification of proteins and is catalyzed in the cell nucleus by enzyme poly(ADP-Ribose)polymerase (PARP). Participation of ADP-ribosylation in various genetic processes is supposed [D'amours et al., 1999, Meli et al., 2003, Zaalishvili et al., 2000]. Therefore, the study of ADP-ribosylation and its biological functions acquires more importance. As for the third level of DNA organization in chromatin, it is determined by the packing of 300Å fibril in the loops, whose ends are attached to nonhistone protein skeleton of cell nucleus (nuclear matrix). Size of topologically independent loops of DNA varies from 5 to 200 kb. There are the data demonstrating the important role of nuclear matrix in the replication, transcription and reparation processes of DNA [Razin, 2001, Nickerson, 2001, Dantzer et al., 2002]. It has been discovered that the enzyme providing the interconversion of topological isomers of DNA through double-strand cleavages, DNA-topoisomerase II and PARP, like many other proteins participating in genetic processes, are mainly localized in the nuclear matrix [Zaalishvili et al., 2000, Dantzer et al., 2002, Glazkov, 1995]. By using purified enzymes it has been demonstrated that PARP ADP-ribosylates DNA-topoisomerase II and inhibits its activity [Darby et al.,1985]. ADP-ribosylation of DNA-topoisomerase II in HeLA cells has been also demonstrated [Scovassi et al., 1993].

The aim of the present work was the study of the role of ADP-ribosylation in the DNA-topoisomerase II activity modulation in the nuclear matrix of a human mononuclear leukocytes and rat brain cortex neurons.

Materials and Methods

The following reagents were used in the given investigation: Tris, β-NAD, SDS, EDTA ("Serva", USA), ATP, phenylmethylsulfonylfluoride (PMSF), thymidine, nonidet P-40, proteinase K, RPMI-1640 medium with L-glutamine, ficoll-400 ("Sigma", USA), SeaKem Gold agarose ("FMC BioProdocts", USA), concatemerized DNA of bacteriophage λ ("BioLabs", USA).

Mononuclear leukocytes were isolated from the heparinized venous blood of healthy donors by centrifugation through the layer of ficoll solution (ρ=1,078g/ml) [Lymphocytes, 1987]. Enriched fraction of neuronal cells (90-95%) was received from the brain cortex of rats weighing 150-170 g [Johnson, Sellinger, 1971, Farooq, Norton, 1978]. Purity of the preparations was controlled with a phase-contrast microscope. Leukocytes and neuronal cells were washed in RPMI-1640 medium and the number of cells was calculated in Goriev Chamber.

Agarose blocks with cells were prepared by the following way: suspensions containing $4x10^6$ cells were centrifuged at 1500 g during 5 min. Sediment was suspended in 50 μL of RPMI having been heated to 37°C and was mixed with equal volume of 1,5% agarose (SeaKem Gold) prepared in the same medium. The mixture was poured into special forms (10X5X1.5 mm) and left there for about 10 min at the temperature of 2°C [Gromova et al., 1995].

For permeabilization and extraction of cells soldered up into the blocks of agarose, the blocks were incubated at 2°C during 1 hr under permanent mixing in buffer solution containing 20 mM Tris-HCl, pH 7,5, 2 mM EDTA, 1 mM PMSF, 0,2% Nonidet P-40, 2M NaCl. The blocks were washed with buffer solution containing 20 mM Tris-HCl, pH 7,5, 50 mM KCl, 10 mM $MgCl_2$ and 0,1 mM EDTA three times, during 30 min each time [Iarovaia, Razin, 1996].

In order to provide ADP-ribosylation and DNA-topoisomerase reaction the blocks were incubated at 25°C during 40 min in buffer solution using for cleaning 1 mM ATP. The reaction was terminated by placing the blocks in stop-buffer containing 0,4 M EDTA, pH 8,0, 1% SDS and proteinase K (0,5 mg/ml) and for protein digestion blocks were incubated in the same medium during 36 hr at 55°C. The blocks were washed in solution of 0,2 M EDTA, pH 8,0 and were used for electrophoresis in the pulse field. Pulse-electrophoresis was carried out in 1% agarose gel (SeaKem Gold) on Bio-Rad CHEF-DR II in the 0,5x Tris-borate-EDTA buffer solution during 20 hr at 14°C at voltage gradient 6 V/cm with the switch time "ramped" linearly from 10 to 90 sec. Concatemerized DNA of bacteriophaghe λ was used as a marker. Gels were stained with the solution of ethidium bromide (0,5 μg/ml) and were photographed through an orange light filter in ultraviolet [Iarovaia, Razin, 1996].

Results and Discussion

For the evolution of the influence of ADP-ribosylation on DNA-topoisomerase activity of the matrix we used the preparation of nuclear matrix obtained by the method being worked out recently [Razin, 2001; Iarovaia, Razin, 1996]. Cells of mononuclear leukocytes and neurons were soldered up into the agarose blocks and the nuclei of permeabilized cells were extracted with high-salt buffer solution, which promotes the removal of extra-matrix DNA-topoisomerase II and PARP and getting the preparation of matrix with intact DNA loops (nucleoid). As DNA-topoisomerase II is localized in the bases of DNA loops the enzyme activity may be determined by its ability to split and religate DNA into regions of fastening loops to the nuclear matrix under certain experimental conditions [Razin, 2001].

Fig. 1 presents the influence of ADP-ribosylation on cleavage of genomic DNA of mononuclear leukocytes with DNA-topoisomerase II of nuclear matrix. The fig. demonstrates that the picture of electrophoretic distribution of DNA in the pulse field changes during the incubation of nucleiods with NAD. NAD at the concentration of 1 mM promotes the intensification of

cleavage of high-molecular genomic DNA. At the same time, the quantity of DNA present in the medium 50-500 kb is increasing, what is accompanied by considerable decrease in the quantity of DNA at the start of the gel and within the zone of compression. As it may be seen from the figure, inhibitor PARP-thymidine [D'amours et al., 1999; Meli et al., 2003; Zaalishvili et al., 2000] at the concentration of 20 mM does not affect the cleavage of genome by DNA-topoisomerase II in the absence of NAD and fully removes the effect of NAD on cleavage. This excludes direct influence of thymidine and NAD on DNA-topoisomerase activity and indicates the influence of ADP-ribosylation on the enzyme activity. The similar picture is observed in case of nucleiods of the enriched neuron fraction (Fig. 2). The quantity of DNA present in the area of 50-150 kb gradually increases with the increasing of NAD concentration from 0,5 mM to 1,5 mM and at the same time the number of DNA at the start of the gel and zone of compression gradually decreases. Inhibitor of PARP, thymidine (20 mM) removes the effect of NAD on DNA cleavage by topoisomerase II that indicates the influence of ADP-ribosylation on the activity of topoisomerase II of nuclear matrix in case of mononuclear leukocytes.

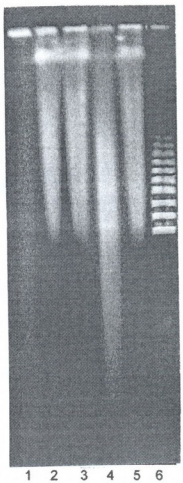

Fig. 1. Influence of ADP-ribosylation on cleavage of genomic DNA by topoisomerase II of nuclear matrix of a human mononuclear leukocytes. Pulse-electrophoregram of agarose gel. 1 – DNA of nucleoids; 2-4 – DNA of nucleoids incubated for 40 min at 25°C in the buffer for carrying out PARP and DNA-topoisomerase reaction (2), in the presence of 20 mM thymidine (3), 1 mM NAD (4), 1 mM NAD and 20 mM thymidine (5), 6 – size markers of bacteriophage λ DNA concatemers (48, 5, 97, 145, 5, … kb.).

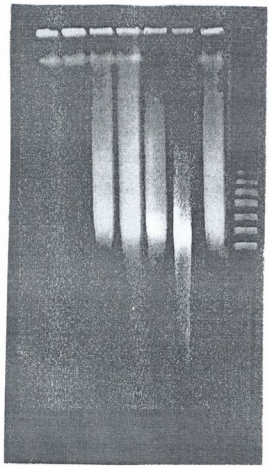

Fig 2. Influence of ADP-ribosylation on cleavage of genomic DNA by topoisomerase II of nuclear matrix of rat brain cortex neurons. Pulse-electrophoregram of agarose gel. 1 – DNA of lysed cells in stop-buffer; 2 – DNA of nucleoids; 3-7 – DNA of nucleoids incubated for 40 minutes at 25°C in the buffer for carrying out PARP and DNA-topoisomerase reaction in the presence of NAD at the concentration of 0, 0.5, 1, 1.5 mM (3-6), in the presence of 1,5 mM NAD and 20 mM thymidine (7), 8 - size markers of bacteriophage λ DNA concatemers (48, 5, 97, 145, 5, … kb.).

As it has been mentioned, during topological isomerization of DNA, topoisomerase II forms temporal double-strand gaps in DNA after what the enzyme religates a molecule. In our case the induction of cleavage of genome of mononuclear leukocytes and neurons during the incubation of cell nucleoids with NAD, is presumably caused by inhibition of the reaction of religation of DNA by ADP-ribosylation. The reason for this is probably ADP-ribosylation of DNA-topoisomerase II. However, it is possible that ADP-ribosylation of any other protein(s) may have allosteric influence on the religation process of DNA by enzyme.

Thus, on the basis of the gained data it may be supposed that PARP associated with nuclear matrix participates in the regulation of the DNA-topoisomerase II activity of the nuclear matrix in eukaryotic cell.

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დნმ-ტოპოიზომერაზა II-ის აქტივობის მოდულაცია ADP - რიბოზილირებით ეუკარიოტული უჯრედების ბირთვულ მატრიქსში

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საქართველოს მეცნიერებათა აკადემიის მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 07.06.2004)

რეზიუმე

დნმ-ის ელექტროფორეზით პულსირებად ველში ნაჩვენებია, რომ ADP-რიბოზილირება ადამიანის მონონუკლეური ლეიკოციტების და ვირთაგვას თავის ტვინის ქერქის ნეირონების ნუკლეოტიდებში ასტიმულირებს დნმ-ის მარყუჟების გახლეჩას მათი ბირთვულ მატრიქსთან მიმაგრების ადგილებში. გამოთქმულია მოსაზრება ADP-რიბოზილირების შესაძლო მონაწილეობაზე ეუკარიოტული უჯრედების ბირთვულ მატრიქსში დნმ-ტოპოიზომერაზა II-ის აქტივობის მოდულაციაში.

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Phytopathology

THE VIRULENCE SPECTRUM OF *ERYSIPHE GRAMINIS F.SP.TRITICI* IN GEORGIA

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(Received May 17,2003)

Abstract

The causal agent of wheat powdery mildew, *Erysiphe graminis f.sp.tritici* had developed annually in Georgia during 2000-2003. Testing of 280 isolates for virulence to 11 single-gene differencially tester lines showed the lack of the virulence to resistance genes Pm2, Pm4a, Pm4b and high contents of virulence genes P1, P3a, P3b, P3c, P5, P8 in population. The frequencies of virulence genes P3d and P6 were 12.8% and 18.5%, respectively. The dominant pathotype was expressed with virulence formula (effective/ineffective host genes): 2, 4a, 4b, 3d, 6/1, 3a, 3b, 3c, 5, 8.

Key words: *Erysiphe graminis f.sp.tritici,* virulence gene

Introduction

Powdery mildew caused by *Erysiphe graminis DC.ex Merat f.sp.tritici em.Marchal* is important disease of wheat. In Georgia it occurs annually on spring and winter wheat cultivars during all vegetation. However, the severity of the disease depends on climatic conditions, on the resistance of a cultivar and on the quantity of the source of infection. It can attack the stem, leaf, spike and in epiphytotic time reduces the crop significantly. The yield losses varies between 10-60% by distribution character of a disease [Mjavanadze, 1972; Zakharova, 1978].

In such situation the role of resistant cultivars especially increases because it provides the depression of pathogen development.

Successful selection depends on the study of virulence structure of the pathogen population. Such kind of investigations have not been yet held in Georgia.

The aim of this work was to determine the virulence genefond of *Erysiphe graminis f.sp.tritici* in Georgia.

Materials and methods

The virulence genefond of *Erysiphe graminis f.sp.tritici* determined on European differential set consisting of 11 near-isogenic lines, each with a single gene, was obtained from Mogens Havmoller, Danish Institute of Agricultural Science (Table 1).

According to the unifed system of designing resistance genes [Ausemus, 1946] the genes resistance to *Erysiphe graminis f.sp.tritisi* (Powdery mildew) are denoted by the symbols Pm1,

Pm2, Pm3a, Pm3b etc., where Pm are the first initials of disease name, 1,2,3 – figures denoted genes locuses, a,b – denoted gene alleles.

Table 1. Differential set (cultivar, isogenic lines) used in a assessment of virulence of *Erysiphe graminis s.sp.tritici*

Isogenic lines,cultivars	Resistance genes
*Axminister CC8	Pm 1
Longbow	Pm 2
*RO 136	Pm 3a
*RO 137	Pm 3b
Sonora CC8	Pm 3c
Vitus Seject	Pm 3d
*Khapli CC8	Pm 4a
Kosack	Pm 4b
Kraka	Pm 5
Holger	Pm 6
*RO 133	Pm 8

* - isogenic lines

In 2000-2003 the populations from four regions of Georgia, namely Mtskheta, Samtredia, Khashuri and Akhaltsikhe were investigated. In general 280 single colony isolates (SCI) were analyzed. Each colony was isolated and grown on seedling plants of the susceptible cv. Hope at 15-20 ^{0}C with 80-100 relative humidity in the greehouse [Krivchenko, 1971; Menzies et all, 1989].

The differentials were raised in trays and inoculated by the single isolates 8 days after planting. Powdery mildew colonies developed in 10 days, at that time a usual assessment of virulence was made for infection type [Mains&Dietz, 1930]. According to the Mains&Dietz's scale the reaction types are as follows: 0 – highly resistant (no mycelium), 1 – resistant (slight to moderate development of mycelium), 2- moderately resistant (a moderate to abundant development of micelium accompanied by a slight production of conidia occurs. Chlorotic or necrotic areas are formed), 3 – moderately susceptible (a moderate to abundant development of micelium accompanied by moderate sporulation occurs), 4 – very susceptible (abundant micelium accompanied by abundant sporulation is developed).

The pathotypes (virulence formula) were recorded according to Green [Green, 1981].

Results

The results of the identification of virulence structure of *Erysiphe graminis f.sp.tritici* population showed that the genefond of virulence is quite representative. Out of the 11 genes analyzed the population consists of 8 virulence genes. The virulence genes p1, p3a, p3b, p3c, p5, p8 were distributed with high frequency – 92.8-100%, virulence of p3 and p6 genes were 12.8%

and 18.5% respectively. The virulence to resistance genes Pm2, Pm4a, Pm4b were not indicated in any isolates (Table 2).

9 pathotype were described according Green. In population the most predominant virulence formula was 2, 3d, 4a, 4b, 6/1, 3a, 3b, 3c, 5, 8. Frequencies of other pathotypes were ranged between 1.3-14.7% (Table 3).

Table 2. Frequencies of virulence genes in *Erysiphe graminis f.sp.tritici* population

Viurlense genes	Frequency, %
p 1	92,8
p 2	0
p 3a	100
p 3b	98,5
p 3c	95,7
p 3d	12,8
p 4a	0
p 4b	0
p 5	92,8
p 6	18,5
p 8	100

Table 3. Distribution of *Erysiphe graminis f.sp.tritici* pathotypes

NN	Pathotipes (virulence formula)	Frequency, %
1.	2,4a,4b,3d,6/1,3a,3b,3c,5,8	56
2.	2,4a,4b,3d/1,3a,3b,3c,5,6,8	14,7
3.	2,4a,4b,6/1,3a,3b,3c,3d,5,8,	9,3
4.	1,2,3d,4a,4b,6/3a,3b,3c,5,8	5,3
5.	2,3d,4a,4b,5,6,/1,3a,3b,.3c,8	5,3
6.	2,3c,3d,4a,4b,6/1,3a,3b,5,8	4
7.	2,4a,4b/1,3a,3b,3c,3d,5,6,8	2,6
8.	1,2,3d,4a,4b,5,6/3a,3b,3c,8	1,3
9.	2,3b,3d,4a,4b,6/1,3a,3c,5,8	1,3

Thus, Georgian population of the causal agent of wheat powdery mildew is quite virulent and due to frequencies of single genes it is similar to North Caucasian population [Anpilogova, Volkova, 2000].

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ხორბლის ნაცრის გამომწვევის *Erysiphe graminis f.sp.tritici* ვირულენტობა საქართველოში

სიხარულიძე ზ., გაბაიძე მ.

მცენარეთა იმუნიტეტის სამეცნიერო კვლევითი ინსტიტუტი

(მიღებულია 17.05.2004)

რეზიუმე

დადგინდა ხორბლის ნაცარის (*Erysiphe graminis f.sp.tritici)* ყოველწლიური გავრცელება ხორბლის ნათესებზე 2000-2003 წლებში. 11 გამძლეობის გენის შემცველი დიპერენციატორების ნაკრებზე 280 იზოლატის ვირულენტობის იდენტიფიკაციამ აჩვენა, რომ პათოგენის პოპულაცია ხასიათდებოდა P1, P3a, P3b, P3c, P5, P8 ვირულენტობის გენების მაღალი შეხვედრის სიხშირით. პოპულაციაში არ დაფიქსირებულა Pm2, Pm4a, Pm4b გამძლეობის გენების შესატყვისი ვირულენტობა. P3d და P6 ვირულენტობის გენების სიხშირე შესაბამისად 12.8% და 18.5% ტოლი იყო. პოპულაციაში დომინირებდა პათოტიპი ვირულენტობის ფორმულით: 2, 4a, 4b, 3d, 6/1, 3a, 3b, 3c, 5, 8.

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Plant Physiology

EFFECT OF HEAVY METALS ON THE PLANT FRUITAGE

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Abstract

Investigation of the effects of the addition of Zn and Cu in nutrient solution on the content of ascorbic acid and sugar in plant leaves, flowers and fruits lead to following conclusions: Zn and Cu inhibit accumulation of ascorbic acid in the leaves of *Chenopodium album, Heracleum, Amarantus speciosus, Ornitogamum pyrenacium* and *Portulaca oleracea.* Cu and Zn has no influence on accumulation of ascorbic acid in flowers and fruits of these plant species. The level of sugar in the leaves and fruits of *Chenopodium album, Heracleum* and *Amarantus speciosus* is significantly decreased under the influence of Zn and Cu, while in *Ornitogamum pyrenacium* and *Portulaca oleracea* the amount of sugar is decreased only in fruits. The effect of Zn and Cu is negative in its nature as long as it results in decrease in the number of fruits. *Urtica urens* shows significant resistance to Cu and Zn.

Key words: plant, Zn, Cu, ascorbic acid, sugar, fruitage

Introduction

Metals represent a general mineral source for plant growth and metabolism. Irrespective to their concentration in plant vegetative and reproductive organs, microelements are crucial for plant nutrition. Plant species differ in the special need in particular microelements [Eliava I., et al., 1992, Bigon D., et al., 1989]. At the same time, heavy metals, crucial for plant development, may turn into dangerous poison, preventing plant species from normal vegetation and fruitage. Progressive pollution of ear and soil and, as a consequence, accumulation of heavy metals in plants - a substantial source of food, is threatening for human health [Clijters H., Van Ashe F., 1985, Raven, P., Johnson G., 1991, Ladigin V., Semionova G., 1993, Nikanorov A., Julidov A., 1991, Obrucheva N., et al., 1993]. Wild plant species are actively used as a source of food all round the world. Wild plant species as a component of human diet are very popular in Georgia, especially in rural regions. However, the role of heavy metals in wild plant species fruitage remains obscure.

Present study was aimed at revealing the effects of zinc and copper on plant fruitage. As long as production of sugar by leaves and concentration of ascorbic acid in plant vegetative and reproductive organs is crucial for fruitage, the concentration of these substances in leaves and flowers was measured as well.

Materials and Methods.

Plant species *Urtica urens, Chenopodium album* and *Heracleum*, as well as *Amarantus speciosus, Ornitogamum pyrenacium* and *Portulaca oleracea* (60 of each species, 30 experimental and 30 control plants) were grown in nutrient solutions [Cherniavina I., et al., 1978] as it is shown in Table 1.

Table 1. Nutrient solution for control and experimental specimen

Salts	Experimental solution g/l	Control solution g/l
$Ca(NO_3)_2$	5.0	5.0
NH_4NO_3	0.2	0.2
$MgSO_4$	0.5	0.5
KCl	0.36	0.36
KNO_3	0.51	0.51
$Fe_2(SO_4)_3$	0.32	0.32
KI	0.028	0.028
Cu	0.001	
Zn	0.001	

Table 2. Content of ascorbic acid in the leaves

plant species	content of ascorbic acid g/ kg in leaves (average of all plants of the given species)								
	at the bottom of plant stem			at the middle			at the top		
	control	Zn	Cu	control	Zn	Cu	control	Zn	Cu
Heracleum	77.6	80	70.3	75.14	68.7	76.1	40	31.1	23.4
Amarantus speciosus	23.3	10	5.1	12.1	5.3	5.4	25.3	22	2.2
Urtica urens	11.4	11	13.2	23.3	19	18.4	12.2	10	10
Chenopodium album	16.4	21.1	22	10.3	9	9	4.1	2.3	3
Portulaca oleracea	70	78	65.3	40.2	44	40.4	16.4	1	4.3
Ornitogamum pyrenacium	23.5	11	6	12	6.4	5.4	20.6	22	23.2

Zinc and copper (0.001 g/l) were added to experimental nutrient solution at the beginning of plant vegetation in March as well as at the start of flower and fruit formation. There were two experimental solutions - one enriched with Zn and another with Cu.

Sugar and ascorbic acid content was measured according to widely accepted methods [Turkina M., Sokolova S., 1971]. Data analysis was undertaken according to methods of mathematical processing [Maksimov V., 1980].

The number of fruits was registered in July and August. Concentration of sugar and ascorbic acid in leaves, flowers and fruits was registered in May, June and July.

Results and discussion.

As it is shown in Table 2, addition of either Zn or Cu to nutrient solution did not influence the concentration of ascorbic acid in the leaves of *Urtica urens*, while content of ascorbic acid was decreased in all other experimental plant species compared with control plants. At the same time, the level of ascorbic acid was decreased in leaves, disposed in the middle and at the top of plant stem, whereas leaves at the bottom comprised the same amount of ascorbic acid compared with control specimen. At the same time, addition of Cu to nutrient solution had mostly strong effect in

Heracleum and *Amarantus speciosus*, while marked decrease of the content of ascorbic acid in *Portulaca oleracea* was registered in plants grown in Zn-rich medium.

No significant difference in the content of ascorbic acid between the flowers and fruits of control and experimental specimen has been revealed (Table 3).

Table 3. Content of ascorbic acid in flowers and fruits

plant species	content of ascorbic acid g/kg average of all specimen in each plant species					
	flowers			fruits		
	control	Zn	Cu	control	Zn	Cu
Heracleum	14.45	13	14	11.45	11.3	12.4
Amarantus speciosus	79.2	68.8	70	17.56	20.1	19.1
Urtica urens	13.66	14.5	18	11.43	12	11.2
Chenopodium album	13.68	10.7	12	25.45	26	27
Portulaca oleracea	88.98	76.4	86.4	38.5	40.1	34
Ornitogamum pyrenacium	52.8	48	49.7	17.34	23.4	20

Table 4. Content of sugar in the leaves and fruits

plant species	subjects	content of sugar g/kg			
		leaves		fruits	
		starch	reducing sugar	starch	reducing sugar
Heracleum	control	3.8	3	3.2	3
	experimental	1	1	1	1
Amarantus speciosus	control	4.67	2.8	4.2	2
	experimental	1	1	1.1	1
Urtica urens	control	2.1	1.05	2.3	2
	experimental	2.1	2	3.1	1.9
Chenopodium album	control	4.1	3.2	4	2.8
	experimental	4.1	4.1	1	1
Portulaca oleracea	control	2.3	1.3	3.2	2.1
	experimental	3.2	2	1	1
Ornitogamum pyrenacium	control	3.8	4.5	2.4	2.4
	experimental	3	5.1	1.9	3

As for content of sugar in the leaves and fruits of examined plants, the level of both, starch and reducing sugar was decreased in *Heracleum, Amarantus speciosus* and *Chenopodium album* while in *Ornitogamum pyrenaceum* and *Portulaca oleracea* the content of sugar was decreased only in fruits compared with control specimen (Table 4). Addition of either Zn or Cu to nutrient solution exerted the same negative effect on the amount of sugar in fruits and leaves of experimental plant species. *Urtica urens* did not show significant alterations in sugar content neither in nutrient solution enriched with Zn, nor in copper-rich medium. The number of fruits was significantly decreased in experimental plant species (with the exclusion of *Urtica urens*) compared with control specimen.

Data obtained lead to following conclusions:

Heavy metals as Zn and Cu inhibit accumulation of ascorbic acid in the leaves of *Chenopodium album, Heracleum, Amarantus speciosus, Ornitogamum pyrenacium* and *Portulaca oleracea*. Cu and Zn has no influence on accumulation of ascorbic acid in flowers and fruits of these plant species.

The level of sugar in the leaves and fruits of *Chenopodium album, Heracleum* and *Amarantus speciosus* is significantly decreased under the influence of Zn and Cu, while in *Ornitogamum pyrenacium* and *Portulaca oleracea* the amount of sugar is decreased only in fruits.

The effect of Zn and Cu is negative as long as it results in decrease in the number of fruits. *Urtica urens* shows significant resistance to Cu and Zn.

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მძიმე მეტალების ზეგავლენა მცენარეთა ნაყოფიერებაზე

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რეზიუმე

ნაჩვენებია, რომ ცინკის ან სპილენძის დამატება საკვებ ხსნარში ამცირებს მცენარეებს: *Chenopodium album, Heracleum, Amarantus speciosus, Ornitogamum pyrenacium* and *Portulaca oleracea* ფოთლებში ასკორბინის მჟავის შემცველობას, მაგრამ ამ მხრივ არ მოქმედებს ყვავილებსა და ნაყოფზე. მეტალების ზეგავლენით მცირდება შაქრის შემცველობა *Chenopodium album, Heracleum* და *Amarantus speciosus* –ის ფოთლებსა და ნაყოფში, ხოლო *Ornitogamum pyrenacium* and *Portulaca oleracea* –ში შაქრის რაოდენობა მცირდება მხოლოდ ნაყოფში. ნაყოფების რაოდენობა მკვეთრად მცირდება ცინკის და სპილენძის ზეგავლენით. ცინკის და სპილენძის უარყოფითი ზეგავლენის მიმართ მდგრადია *Urtica urens.*

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Zoology

ORNITOFAUNA OF SATAPLIA RESERVE AND THEIR DISTRIBUTION BY BIOTOPS

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Abstract

Ornitofauna of Sataplia Reserve was studied by route method. In the reserve territory 39 species and subspecies of birds are spread. Among them 23 species and subspecies are residing and 15 – nesting. The birds of reserve belongs to 8 order, 21 families and 23 genus. Passeriformes order is distinguished with the biggest number of species – 27 species and subspecies. In the near-forest shrubs 19 species and subspecies are spread, in the mixed leaf-bearing forests – 26 and in the alder forest – 20. With the development of reserve forest species compositional and quantitative changes are expected.

Key Words: Reserve, route method, *Passeriformes, Falciniformes, Piciformes.*

Introduction

The biodiversity of Caucasus is the result of very complicated relief of this territory and climatic peculiarities. In the view of origination of animate nature the heterogenity of Caucasus region the is most evident in Georgia. West Georgia (Colchi) is the most interesting for relicts of flora and fauna characterizing the end of Miocene and Pliocene. Archaic spectrum of Georgian flora and fauna is not repeated in any other place [Zhordania et al., 1999, Zhordania, 2002]. So, study of the nature of Caucasus and especially Georgia is very important.

The goal of our research is characterization of ornitofauna of Sataplia State Reserve and their distribution in different biotopes.

Materials and Methods

Ornitofauna of 146,5 ha area of Sataplia Reserve (the whole territory of which is 354 ha) was studied by route method [Mikheev, 1975]. The territory was divided into three different biotopes: near-forest shrubs, mixed leaf-bearing forest and alder forest. Schedule was carried out mainly in the nesting period. Every route was gone over twice a day: 7-11 a.m. and 18-20 p.m., at proper weather conditions (17-24^0 C, windless and unclouded day).

Results and discussion

The area of reserve is 354 ha, we have researched 146,5 ha (Table 1).

Table 1. Distributions of different biotopes in Sataplia reserve.

№	b i o t o p e	researched area (ha)	width of liniar transsection (m)	scheduled area (ha)
1	near-forest shrubs	15	20	30
2	mixed leaf-bearing forest	125	20	250
3	alder forest	6,5	20	13
4	total	146,5	—	293

The studies show that in reserve territory 38 species and subspecies of birds are distributed. Among them 23 species and subspecies is residing and 15 species – nesting Paseriformes order (27 species and subspesies) is distinguished with biggest number of species. On the second place Piciformes and Falciniformes (each with 3 species and subspecies). Then comes Caraciiformes (with 2 species). Microdiformes, Caprimulgiformes, Strigiformes and Cuculiformes are represented with one species each (Table 2).

In the near-forest shrubs 19 species and subspecies of birds are distributed: *Sylvia communis icterops, Lanius cristatus kolylini, Phylloscopus collybitus, Carduelis carduelis brevirostris, Fringilla coelebs solomkoi, Aegithalos caudatus major, Parus ater, Turdus merula merula, Troglodytes troglodytes troglodytes, Upupa epops epops, Garrulus glandarius krynicki, Parus major major, Phoenicurus phoenicurus, Luscinia luscinia, Passer domesticus domesticus, Motacilla cinerea cinerea, Apus apus apus, Hirundo rustica rustica.*

Table 2. Ornitofauna of Sataplia Reserve.

N	Order	Family	N	Species	residing or nesting
1	2	3	4	5	6
1.	Falciniformes	Accipitridae	1.	*Accipiter gentilis*	residing
			2.	*caucasicus*	"
			3.	*Accipiter nisus nisus*	"
				Buteo buteo	
				menetriesi	
2.	Cuculiformes	Cuculidae	4.	*Cuculus canorus*	nesting
				canoru	
3.	Strigiformes	Strigidae	5.	*Strix aluco*	residing
				wilkonskii	
4.	Caprimulgiformes	Caprimulgidae	6.	*Caprumulgos*	nesting

				europaeus meridionalis	
5.	Microdiformes	Apodidae	7.	*Apus apus apus*	"
6.	Caraciiformes	Meropidea	8.	*Merops apiaster*	"
		Upupidae	9.	*Upopa epops epops*	"
7.	Piciformes	Picidae	10.	*Picus viridis*	residing
			11.	*karelin*	"
			12.	*Dendrocopos maior tenuirostris*	"
				Dendrocopos medius caucasicus	
8.	Passeriformes-	Hirundinidae	13.	*Hirundo rustica rustika*	nesting
		Matacillidae	14.	*Motacilla alba alba*	"
			15.	*Motacilla cinerea cinerea*	residing
		Laniidae	16.	*Lanius collurio kobylini*	nesting
		Troglodgtidae	17.	*Troglodytes troglodytes troglodytes*	residing
		Turdidae	18.	*Turdus merula merula*	"
			19.	*Turdas viscivorus viscivorus*	nesting
			20.	*Phoenicurus phoenicurus*	"
			21.	*Saxicola torquata*	"
			22.	*Luscinia megrrhyndas*	"
			23.	*Erithacus rubecula caucasicus*	"
		Sylviidae	24.	*Phylloscopus collybitus*	"
			25.	*Sylvia atricapilia dammaholz*	"
			26.	*Sylvia communis icterop*	"
		Paraxornithidae	27.	*Aegithalos caudatus major*	residing
		Paridae	28.	*Parus major major*	"
			29.	*Parus ater*	"
			30.	*Parus coeruleus satunini*	"
		Sittidae	31.	*Sitta canadensis kruperi*	"
		Emberizidae	32.	*Emberiza cia*	"

				prageri	
			33.	*Chloris choris bilkewitschi*	"
			34.	*Pyrrhula pyrrhula rossikowi*	"
			35.	*Carduelis carduelis brevirostris*	"
			36.	*Fringilla coelebs solomkoi*	"
			37.	*Passer domesticus domesticus*	"
		Corvidae	38.	*Corvus corone sharpii*	"
			39.	Garrulus glandarius krynicki	"

By researches carried out in 1969 14 species were described. 5 species - *Passer domesticus domesticus, Turdus merula merula, Motacilla cinerea cinerea, Apus apus apus*, and *Hirundo rustica rustica* – were added after 1969. In this complex Sylvia communis is dominant.

In the mixed leaf-bearing forest which is mainly composed by oriental hornbeam forest, beech forest, hawthorn, rhododendron, box-tree, bilberry bush, etc. are spread 26 species and subspecies of birds: *Fringilla coelebs solomkoi, Phylloscopus collybitus, Turdus merula merula, Parus major major, Garrulus glandarius kryniki, Parus ater, Erithacus rulecula caucasicus, Aegithalos caudatus major, Picus viridis karelini, Sitta canadensis kruperi, Troglodytes troglodytes troglodytes, Strix aluco wilkonskii, Pyrrhula pyrrhula rossikowi, Dendrocopos major tenuirostris, Sylvia atricapilla dammholzi, Corvus corone cornix, Buteo buteo menetriesi, Accipiter gentillis caucasicus, Accipiter nisus nisus, Cuculus canorus canorus, Chloris chioris bilkewitschi, Caprimulgus europaeus meridionalis, Motacilla alba alba, Emberiza cia prageri, Saxicola torquata.*

In this biotope density of population is average. *Fringilla coelebs solomkoi, Phylloscopus collybius* and *Turdus merula merula* are dominant species and they compose 41,7% of whole birds population. By 1969 schedule in this biotope there were 21 species and by new data the number of species is increased to 26 species.

In alder forest were found 20 species and subspecies: *Parus ater, Turdus merula merula, Parus major major, Accipiter nisus nisus, Strix aluco Wilkonskii, Sylvia atricapilla dammholzi, Fringilla coelebs solomkoi, Garrulus glandarius krynicki, Aegithalos caudatus major, Troglodytes troglodytes troglodytes, Pyrrhula pyrrhula rossikowi, Sitta canadensis kruperi, Corvus corone cornix, Dendrocopos major tenuirostris, Cuculus canorus canorus, Buteo buteo menetriesi, Parus coeruleus satunini, Merops apiaster, Dendrocopos medius caucasicus, Turdus viscivorus.* In this biotope by 1969 schedule were described 14 species, today – 20 species of birds. 6 species - *Silvia atricapilla dammholzi, Dendrocopos major tenuirostris, Parus coeruleus satunini, Merops superciliosus persicus, Dendrocopos medius caucasicus, Turdus viscivorus viscivorus* – were added with the development of the forest.

Thus, Sataplia Reserve is mainly resided with song-birds and insectivorous birds. With the dynamics of flora the species composition and number may be changed.

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სათაფლიას სახელმწიფო ნაკრძალის ორნითოფაუნა და მათი ბანაწილება ბიოტოპების მიხედვით

გორდაძე ე., ჟორჟოლიანი ც.

ბიოლოგიის კათედრა, ქუთაისის აკაკი წერეთლის სახელობის სახელმწიფო უნივერსიტეტი

(მიღებულია 10.05.2004)

რეზიუმე

ნაკრძალის ტერიტორია შეადგენს 354 ჰექტარს. მარშუტული მეთოდით გამოკვლეულია 146,5 ჰექტარი. ნაკრძალის ტერიტორიაზე გავრცელებულია სულ 38 სახეობის და ქვესახეობის ფრინველი. აქედან 23 სახეობა და ქვესახეობა მობინადრეა, 15 სახეობა მობუდარი. ნაკრძალის ფრინველები ეკუთვნის 8 რიგს, 21 ოჯახსა და 23 გვარს. სახეობათა ყველაზე მეტი რაოდენობით გამოირჩევა ბელურასნაირების რიგი – 27 სახეობა და ქვესახეობა. ტყისპირა ბუჩქნარში გავრცელებულია 19 სახეობა და ქვესახეობა, შერეულ ფოთლოვან ტყეში - 26, ხოლო მურყნარში – 20. ნაკრძალში ტყის განვითარებისთან ერთად მოსალოდნელია ფრინველთა სახეობრივი და რაოდენობრივი ცვლილებები.

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Zoology

PECULIARITIES OF AMINO ACID CONTENT OF BLOOD PLASMA PROTEINS IN TAXONOMICAL AND ECOLOGICAL POINT OF VIEW

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Abstract

Amino acid content of blood plasma proteins of high and small mammals and birds was studied. Amino acid concentrations of animals integrated in considerably low taxonomic units (populations, subspecies, species, genus) are phylogenetically stable and conservative. The differences revealed between the animals united in high taxonomic units (family, class). From birds to high mammals the tendency of consequent increase of concentrations of amino acids – Val(V), Tre(T), Cys(C) and visa versa the tendency of decrease of concentrations of amino acids His(H) and Met(M). It was established that pollution of animals habitat causes the increase of Arg(R) concentration.

Key words: taxonomy, amino acids, blood plasma proteins, mammals, birds.

Introduction

To determine and compare the amino acid concentrations of blood plasma proteins of animals belonging to different taxons for establishment of systematical parameters is of interest [Vishniakov et al., 1978; Khristova, 1977; Tajieva, Akhmetov, 1980; Lagodiuk et al., 1983; Pisera, Ivanova, 1988; Ratiani, 1992]. To ascertain the taxonomical and ecological peculiarities and connections of animals, various species and populations of birds and high and small mammals spread in Georgia were studied.

Materials and methods

From small mammals were studied: 24 specimen of *Microtus socialis pal* of Gori and Mtskheta regions; *Apodemus sylvaticus* – 23 specimen of Gori, 26 of Rustavi, 18 of Borjomi and 14 of Mtskheta populations; 30 specimen of cattle; 33 specimen of *Sus scrofa*; 60 of domestic pig; 30 of *Sus scrofa* and domestic pig's hybrids. From birds 14 specimen of *Columbia livia* and 30 of *Galus galus*. Totaly 302 adult animals were investigated. Data were obtained and treated by Moore and Stain method [Moore, Stain, 1963]. Analyzator AAA T-339 was used.

Results and discussion

The first series of experiment was carried out on different species of pigs. Data obtained from 19 specimen of *Sus scrofa attila*, 11- *Sus scrofa scrofa*, 3 – *Sus scrofa nigripes*, 30 – domestic pigs, 30 – hybrids of *Sus scrofa* and domestic pig are presented in table 1. Different species of *Sus scrofa*, domestic pig and their hybrids don't differ from each other by amino acids concentrations of blood plasma proteins statistically truly. The concentrations of studied 17 amino acids are practically identical. The indices of similarities (r) of studied parameter of above mentioned species vary from 0,9385 to 0,9640. With the high concentration are noted Glx(E) (14,18-14,26%) and Asx(D) (11,03-1127%) and with low concentration amino acids Met(M) (1,00-1,08%) and Ile(I) (1,82-1,90%).

Table 1. Amino acid concentrations (%) of blood plasma proteins of domestic and wild pigs and their hybrids

amino acid	Wild boar			Domestic pig		Hybrids
	Sus scrofa attila n=19	*Sus scrofa scrofa* n=11	*Sus scrofa nigripes* n=3	domestic pig (Landrase) n=30	domestic pig (coarse white) n=30	F_1 n=30
Asx(D)	11,19	11,18	11,27	11,20	11,03	11,15
Tre(T)	4,50	4,61	4,59	4,52	4,60	4,56
Ser(S)	5,30	5,29	5,31	5,20	5,33	5,28
Glx(E)	14,18	14,26	14,20	14,24	14,14	14,19
Oro(P)	4,53	4,48	4,51	4,58	4,50	4,52
Cys(C)	2,60	2,52	2,59	2,57	2,55	2,53
Gly(G)	3,89	3,90	3,95	3,91	4,00	3,97
Ala(A)	4,47	4,55	4,40	4,53	4,57	4,50
Val(V)	4,12	4,10	4,08	4,02	4,14	4,11
Met(M)	1,06	1,00	1,08	1,01	1,05	1,04
Ile(I)	1,83	1,85	1,82	1,89	1,90	1,87
Leu(L)	8,40	8,59	8,51	8,44	8,42	8,50
Tyr(Y)	9,06	8,95	8,93	9,00	9,02	9,00
Phe(F)	4,21	4,24	4,17	4,20	4,22	4,20
His(H)	7,66	7,56	7,62	7,67	7,58	7,63
Lys(K)	8,81	8,83	8,89	8,87	8,85	8,83
Arg(R)	4,19	4,09	4,08	4,15	4,10	4,12

To generalize received data in the view of taxonomy second series of experiment was carried out on two species of mouse – usual forest mouse (*Apodemus sylvaticus*) and mouse with yellow spotted neck. These species reside in the same places but phenotypically they differ. In scientific literature about mammals systematization there are different opinions, some scientists consider them as one species – *Apodemus silvaticus* and others as different subspecies of *Apodemus silvaticus*. By the methods of hybridization and genetics neck-spotted and unspotted specimen of Georgian forest mouse were considered as one species - *Apodemus silvaticus* [Ratiani, Tskipurishvili, 2002].

Amino acid content of 47 usual and 34 neck-spotted forest mice were studied and compared. Differences between them by amino acid content were not observed statistically truly (Table 2.). Comparison of *Apodemus silvaticus* different populations with *Microtus socialis pal* revealed that index of similarity varies from r = 0.8903 to 0,9450. Amino acids Glx(E) (14,46-

12,68%) and Asx(D) (11,47-1052%) are noted with high concentrations. Ile(I) (1,30-2,47%) and Met(M) (1,60-2,32%) – with low concentrations.

Table 2. Amino acid concentrations (%) of blood plasma proteins of *Apodemus sylvaticus and Microtus socialis pal.*

amino acid	*Apodemus sylvaticus* population				*Microtus socialis pal.* n=24
	Gori region n=23	Rustavi n=26	Borjomi region n=18	Mtskheta region n=24	
Asx(D)	11,04	10,64	11,13	11,47	10,52
Tre(T)	3,12	3,41	3,36	3,85	3,56
Ser(S)	5,68	5,90	6,03	8,50	6,73
Glx(E)	14,13	13,94	14,46	12,68	13,33
Pro(P)	5,87	4,92	3,59	4,30	7,91
Gly(G)	3,21	3,73	3,24	5,18	3,07
Ala(A)	7,01	5,35	6,68	5,95	6,62
Cys(C)	2,02	1,93	2,00	1,60	2,25
Val(V)	2,42	3,12	2,69	2,45	3,30
Met(M)	1,85	1,61	2,10	2,32	1,60
Ile(I)	1,30	1,47	1,33	2,47	1,71
Leu(L)	7,65	7,81	9,13	9,20	8,38
Tre(T)	6,22	5,42	5,09	5,16	5,60
Phe(F)	7,41	7,65	7,06	4,42	5,77
His(H)	9,57	8,53	9,68	9,04	7,00
Lys(K)	8,14	9,12	8,78	6,68	8,36
Arg(R)	3,26	5,45	3,65	5,23	4,20

Table 3. Amino acid concentrations(%) of blood plasma proteins of birds and mammals.

amino acids	*Columba livia* n=14	*Gallus gallus* n=30	*Apodemus sylvaticus* n=41	*Microtus socialis pal* n=24	*Sus scrofa* n=3	Domestic pig n=30	Cow n=30
Ala(A)	5,78	4,01	7,01	6,62	4,47	4,57	3,87
Arg(R)	4,40	6,94	3,26	4,20	4,19	4,10	3,67
Asx(D)	10,43	12,00	11,14	10,52	11,19	11,03	11,27
Gly(G)	4,07	4,20	3,21	3,07	3,89	4,00	2,58
Glx(E)	13,11	14,18	14,13	13,33	14,18	14,14	12,97
Val(V)	2,11	2,16	2,42	3,30	4,12	4,14	4,49
Tyr(Y)	7,19	7,74	6,22	5,60	9,06	9,02	10,46
Ile(I)	1,48	1,67	1,30	1,71	1,83	1,90	1,62
Leu(L)	6,76	6,46	7,65	8,38	8,40	8,42	7,87
Lys(K)	6,04	6,98	8,14	8,36	8,81	8,85	8,68
Met(M)	3,54	2,46	1,85	1,60	1,06	1,05	1,08
Pro(P)	7,23	4,12	5,87	7,91	4,53	4,50	4,54
Ser(S)	6,84	6,33	5,68	6,73	5,30	5,33	7,35
Tre(T)	3,09	3,35	3,12	3,56	4,50	4,60	5,75
Phe(F)	6,25	6,61	7,41	5,86	4,21	4,22	3,65
Cys(C)	1,35	1,44	2,02	2,25	2,60	2,55	3,94
His(H)	10,43	9,35	9,57	7,00	7,66	7,58	6,21

Gori and Borjomi regions are ecologically considerably pure, Rustavi region is polluted. Mtskheta region specimen were caught after 2 month of treatment of the plot with pesticides. Study

of 17 amino acid concentrations has shown that concentration of Arg(R) of Gori and Borjomi populations were nearly the same – 3,26% and 3,65%, but these parameters of Rustavi and Mtskheta populations were increased 1,5 times and were – 5,45% and 5,23%. Analogous results were received while studying the milk – artificial increase of cobalt content in cows fodder (1 kg fodder/1,50 mg Co) increase Arg(R) concentration in milk by 12,5% [Lisenko, 1991].

Artificial pollution of Mtskheta region provoke not only Arg(R) concentration increase, but notably increase concentrations of Ser(S) (8,50%) and Gly(G) (5,18%) and decrease of Phe(F) (4,24%). So, it should be suggested that level of pollution of region positively correlates with concentration of Arg(R).

To compare higher taxons, in the next series of experiment amino acid concentrations of mammals and birds united in higher systematical groups were studied (table 3). Amino acid concentrations of birds - *Columba livia* and *Galus galus* - don't distinguished from each other statistically truly. Analogous parameters have small mammals (*Apodemus sylvaticus* and *Microtus socialis pal*) and high mammals (*wild and* domestic pigs, cow.).

Total concentrations of amino acids – Arg, Val, Ile, Leu, Lys, Met, Tre, Phe, His are in: *Columba livia*– 44,00%, *Galus galus*- 45,98%, *Apodemus sylvaticus* – 44,72%, *Microtus socialis pal* – 43,97%, *Sus scrofa* – 44,78%, domestic pig- 44,86%, Cow - 43,02%. So, the studied animals are characterized with similar concentrations of amino acids of blood plasma proteins.

Comparison of 7 species by 17 amino acids concentrations have shown that index of similarities varies from r = 0,8133 to 0,9450. By the highest concentration are characterized Glx (12,97-14,18%), Asx (10,43-12,00%) and by low concentration – Ile (1,30-1,90%).

It is worth to note the tendency of increase and decrease of some amino acid concentration of researched animals. The concentration of Val in *Columba livia*'s blood plasma proteins was 2,11%, in *Galus galus*- 2,16%, in *Apodemus sylvaticus* – 2,42%, in *Microtus socialis pal* – 3,30%, in *Sus scrofa* – 4,14%, in domestic pig- 4,14%, in Cow - 4,49%. Val concentration in high mammals was 2 times more than in birds. With the same tendency of increase of amino acid concentration are characterized Tre (3,09-5,75%), Tir (7,19-10,46%). With the tendency of decrease of concentration are noted amino acid His (10,43-6,21%); concentration of Met in plasma proteins is 3 times high (3,54%) than in high mammals (1,05-1,08%).

Thus, between the animals united in low taxonomic groups (species, genus) differences of blood plasma proteins amino acid concentrations are not observed. The differences are revealed between the animals united in high taxonomic groups (family, class). From birds to high mammals the tendency of consequent increase of concentrations of amino acids – Val, Tre, Cys, and the tendency of decrease of His and Met was noted.

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ძუძუმწოვრებისა და ფრინველების სისხლის პლაზმის ცილების ამინმჟავური შემცველობის თავისებურებანი ტაქსონომიური და ეკოლოგიური თვალსაზრისით.

რატიანი ჯ., ბეგელაური ხ., ნადირაშვილი მ.

საქართველოს მეცნიერებათა აკადემიის ზოოლოგიის ინსტიტუტი

(მიღებულია 07.06.2004)

რეზიუმე

შესწავლილია მსხვილი და წვრილი ძუძუმწოვრებისა და ფრინველების სისხლის პლაზმის ცილების ამინმჟავური შემცველობა. შედარებით დაბალ ტაქსონომიურ ერთეულებში (პოპულაცია, ქვესახეობა, სახეობა, გვარი) გაერთიანებული ცხოველთა ჯგუფების სისხლის პლაზმის ცილებში ამინომჟავური კონცენტრაცია ფილოგენეტიკურად მდგრადი და კონსერვატულია. განსხვავება ვლინდება მაღალ ტაქსონომიურ ერთეულებში (ოჯახი, კლასი) გაერთიანებულ ჯგუფებს შორის. ფრინველებიდან მსხვილ ძუძუმწოვრებამდე აღინიშნება ვალინის, ტრეონინის, ცისტეინის კონცენტრაციის თანმიმდევრული ზრდის ტენდენცია და პირიქით, აშკარაა ჰისტიდინისა და მეთიონინის კლებადობის ტენდენცია. დადგენილია, რომ ცხოველთა ადგილსამყოფელის დაბინძურება იწვევს სისხლის პლაზმის ცილებში არგინინის კონცენტრაციის რამდენადმე გაზრდას.

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Letter to Editor

TENDENCY OF CO_2 GROWTH IN ATMOSPHERE AND ITS EFFECT ON FORESTS ECOSYSTEMS OF GEORGIA

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Carbon Dioxides, one of the greenhouse gases rate of change between atmosphere and phytocenosis considerably stipulate maintenance of total balance of CO_2 in atmosphere.

According to the global scale meteorological observations carried out from the middle of the 19th century till now, quantity of atmospheric CO_2 increased by 25%, mainly due to the effect of anthropogenic factor. Respectively, average annual temperature raised within 0,5-1^{0}C. Mathematical modeling showed that in case of CO_2 doubling in the middle of current century, 1,5-4,5^0 C global climate warming is expected which may affect ecological stability of biota [Modeling Climate Change, 1995]. Therefore, limitation of emission of anthropogenic greenhouse gases and strengthening of intensity of natural sources absorbing these gases (particularly forests), take significant place in the list of main requirements of the Framework of Climate Change Convention of the UN (FCCC). Attention is focused on assessment of vulnerability of natural ecosystems to climate changes and development of adaptation strategy [Gzirishvili T.,1997].

Forests cover 30% of the land (57-60x10^8 ha). About 90% of carbon accumulated in the land biota and 60% of primary bio-production come to the forests. Approximately 1-2 million km^2 will be covered with forests in order to stabilize Carbon Dioxide quantity in the Earth's atmosphere [Coombs J., et al, 1986].

The growth of CO_2 concentration within environment increases photosynthesis activity i.e. the main physiological process of the plant. Increase of CO_2 concentration by 1% in the atmosphere rises the photosynthesis rate by 0.5% [Carbon Dioxide in Atmosphere, 1987]. Photosynthetic activity of plant cover in a number of regions of the earth has increased by 10% in comparison to the beginning of the 20th century. Recently it was shown, that doubling and tripling of CO_2 quantity within closed system of atmosphere cause the increase of bio-productivity of agricultural forests of *Populus hybrida* up to 60% and 82% correspondingly [Rosensteil T., et al., 2003]. However, CO_2 concentration growth and accelerated warming tendency may cause a number of negative effects.

Calculations showed [Modeling Climate Change, 1995] that during the next 50 years average rate of temperature rise at CO_2 concentration doubling may reach to 0,2 ^{0}C in a decade, which twice exceeds possible range (0,1 ^{0}C) of natural ecosystems adaptation in a decade [Gzirishvili T.,1997]. Rise of the temperature up to 0,2^{0}C in a decade means the change of latitude by the rate - 10 km/year.

Global warming process can not equally influence on ecosystems existing in different zones of the earth, "loser" and "winner" regions will appear. Precipitation quantity as well as soil humidity will fall down in temperate zone, especially in dry regions together with the rise of temperature. Probability of forests vulnerability will considerably increase. Climate warming for

the north plant cover may result in a positive effect. Soil thickness and draining will grow up along with glacier melting. Photosynthesis intensity and biomass accretion will raise and vegetation period will be extended too. Forests are expected to be expanded to the North and species composition of dendroflora to be changed.

Hence, forests development process depends on regional peculiarities of global warming. Regional models of expected climate changes in the nearest 50-100 years with net element of about 15 km minimum size are being developed in a number of countries.

Expected regional climate changes and vulnerability of Georgian forests.

At present forests cover 39,6% ($2,75x10^6$ha) of the territory of Georgia. 99,3 % of them belongs to the first group forests. Majority (72,8%) of forests grow at 500 m above sea-level. Total wood supply equals to $434x10^6$ m^3. Diversity of dendroflora is represented by approximately 400 of wildly growing arboreal species. Out of them 61 species are Georgian endems and 43 - Caucasus. More than 3600 arboreal plants are introduced. Deciduous forests occupy 83,6% of the territory, coniferous - 16,4%. In western Georgia forest expand area (50,9%) significantly exceeds the area in eastern Georgia (30%). There are 6 vertical belts in east Georgia and 4 - in west.

Effect of regional phenomenon of global climate changes on forest development process in Georgia until recently was not actually studied. Dynamics of separate elements (temperature, precipitations) of climate and their territorial variations in previous century were demonstrated in National Research Center of Hydro-Meteorological Department of Georgia by applying empirical-statistical model [Elizbarashvili E., et al., 1997].

Gradations of temperature changes during 90 years (1906-1995) in cold and warm periods on average reached to $-0,03^0$C/10years and $+0,05^0$C/10years correspondingly. Such rates of temperature changes are considerably behind of marginal rate of plant ecosystems temperature adaptation, which is $+0,1^0$C/10years.

The features of atmospheric precipitation changes in 1938-1990 are rather variable and complicated. The range of total precipitation changes within warm period are rather high in some regions of Georgia: –5 +3 %/10 years. This factor might have affected forests development.

Pursuant to one of hypothesis [Modeling Climate Change, 1995] in the nearest 50 years rise of temperature from 1^0C to $1,5^0$C is expected in Transcaucasus and Georgia, which may extensively increase frequency of climatic anomalies and reduce ecophysiological stability of forests. Diversity of plant species and photosynthesis activity will be diminished. Tendency of temperature rise will constrict alpine belt and change the area of a number of species of Georgian dendroflora, as well as character of vertical distribution. According to the latest data [Nakhutsrishvili G., et al., 2004] plant cover changes tendency on the upper border of the forest in the Central and Lesser Caucasus has been revealed.

Agricultural forests as potential source of CO_2 sequestration from atmosphere.

Development of agricultural forests of Georgia by intensively grown introduced plant species in the area with damaged forests effected by anthropogenic and other factors which aboriginal species restoration is impossible has been assessed. Depending on principles of ecologically sustainable development, for strengthening of CO_2 sequestration from atmosphere, it is necessary to plant multifunctional agricultural forests near industrial centers.

Due to high rates of expected rise of CO_2 concentration and temperature of atmosphere rapidly growing tree plants of high photosynthesis activity and productivity, which give required effect in a short time (20-30 years), must be used in order to increase intensity of CO_2 absorbtion.

Among rapidly growing tree plants *Eucalyptus globulus* and *Populus hybrida,* as well as *Robinia pseudoaccacia* are distinguished with high rate of growth activity in Georgia. *Robinia pseudoaccacia* and *Populus hybrida* are notable for relatively wide eco-climatic range, they grow both in regions of eastern and western Georgia. Taking into account biological peculiarities of saplings and agrosilviculture standards plants maintain high growth rates within the whole vegetation period and give rather high annual profits of wood. Besides, they have high reproduction ability. Roots and cut trees give huge growth of sprouts with high activity of natural revival which minimize expenses of looking after forest crops [Cherkezishvili T., 1998].

Average annual profits of wood of *Robinia pseudoaccacia*, *Eucalyptus globulus* and *Populus hybrida* plants grown in optimal conditions and quantity of accumulated C and CO_2 are given in table 1. Age of young plants, dry woods average density and supply are also shown in the table. In calculations performed we used data of scientific literature [Coombs S., et al., 1986, Cherkezishvili T., 1998, William H., et al., 1981].

Unfortunately, in 90s of the 20th century plantation works significantly reduced against the background of illegal forest cutting. In 1991 -1996 in comparison with 1986-1990 their number catastrophically diminished approximately by 70%. Therefore, development of poly-functional agro-forests in order to fill resources caused by forest cutting and expected climate changes nowadays are important. Development of projects of optimal plantation and correct management of exploitation may become agricultural forests economicaly profitable which facilitate to cover costs of plantation, as well as natural resources of the forests will be saved.

Table 1. Average annual rate of woods profits and accumulated C and CO_2 in *R.Pseudoaccacia, Eucalyptus sp.* and *Populus hibrida* plants grown in optimal conditions

Species	Age of plants (yr)	Wood supply (m^3/ha)	Average density of dry wood ρ(t/m^3)	Average accretion of wood M (m^3/ha.yr)	Dry matter accretion (t/ha.yr) A= ρxM	Accumulation of C t/ha.yr B=0,45x A	Accumulation of CO_2 (t/ha.yr.) C=B x 44/12
Eucalyptus globulus.	35	1200	0,7	34	24	11	40
Robinia pseudoaccacia	30	750	0,75	25	19	8,5	31
Populus hybrida	35	1000	0,45	28	13	6	21

Hence, on the basis of the existing information we may come to the following conclusion:

- temperature (-0,03^{0}C/10yr - +0,05^{0}C/10yr) and precipitation (-5%/10yr - +3%/10yr) gradations revealed in Georgia during the last century did not considerably affect general condition of forests though, plant transformation tendency on upper border of Central and Lesser Caucasus is mentioned today.
- To assess the impact of climatic changes on Georgian forests, wide network of ecoclimatic monitoring will be established to reveal plant cover inclinations in different regions and eliminate them.
- Rapidly growing wood species with wide ecological range will be applied in order to increase dedroflora adaptation to the climatic changes.

– In the areas where restoration of natural forest is impossible, to carry out plantation of agro-forests by using rapidly growing and high productive plants (*Eucalyptus globulus, Populus hybrida, R. Pseudoaccacia* and etc), which accumulate big quantity of CO_2 (21-40 t CO_2 /ha.yr) within a short period of time (20-30 years), along with other means performed for the reduction of CO_2 quantity in atmosphere.

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ატმოსფეროში CO_2 -ის რაოდენობის ზრდის ტენდენცია და მისი გავლენა საქართველოს აგროეკოლოგიაზე

თარხნიშვილი გ., ფხაჭაიშვილი ს., ლოლაძე ტ., ჯაიანი გ., მგალობლიშვილი მ., ხეცურიანი ნ., ნაჭყებია კ., სანაძე გ.

ფოტოსინთეზის პრობლემური სამეცნიერო-კვლევითი ლაბორატორია, ილ. ჭავჭავაძის სახელობის თბილისის სახელმწიფო უნივერსიტეტი

(მიღებულია 07.06.2004)

რეზიუმე

განხილულია კლიმატის ცვლილებისადმი დენდროფლორის ადაპტაციის გასაზრდელად სწრაფმზარდი და ფართო ეკოლოგიური ამპლიტუდის მქონე ტყის ახალი ჯიშების გამოყენების აქტუალობა. განხილულია ატმოსფეროში CO_2-ის რაოდენობის ზრდის შესამცირებლად, სხვა ღონისძიებებთან ერთად, იქ, სადაც ბუნებრივი ტყე აღარ აღდგება, აგროტყეების გაშენება სწრაფმზარდი და მაღალპროდუქტიული ტყის კულტურების გამოყენებით (*Eucalyptus globulus, Populus hybrida, R. Pseudoaccacia* and etc). ნაჩვენებია, რომ ასეთი აგროტყეები დროის საკმაოდ მოკლე მონაკვეთში (20-30 წელი) CO_2-ის დიდი რაოდენობით აკუმულაციას ახდენენ (21-40 ტ/ჰა.წ.).