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Biochemistry

COMPARATIVE CHARACTERIZATION OF TEA LEAF AND WALNUT GREEN HUSK PHENOL OXIDASES

OMIADZE N., MCHEDLISHVILI N., GULUA L., ZAMTARADZE R., ABUTIDZE M., TSIKLAURI G.

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(Received August 6, 2007)

Abstract

Phenol oxidases from tea *(Camellia Sinensis L.)* leaf and walnut *(Juglans regia L.)* green husk were isolated. Monophenolase (Monophenol Monooxygenase EC.1.14.18.1) and catechol oxidase (CO; EC 1.10. 3.1) activities of the enzymes were studied. It was shown that phenol oxidase activity of the enzymes from both plants reached the maximum in July-August. Tea leaf and walnut green husk monophenolases exhibited maximum activities at pH 7.1 and 6.9 respectively. Kinetics of monophenolase reactions did not follow Michaelis-Menten equation.

Key words: tea leaf, walnut green husk, monophenolase, catechol oxidase, kinetics

Introduction

Phenol oxidases are widely distributed in most of higher plants [Mayer, 1987; Fenoll et al., 2002]. Phenol oxidases catalyse two different reactions: monophenol hydroxylation into o-diphenols (monophenolase activity) and dehydrogenation of o-dioxysubstituted polyphenols (catechol oxidase activity) [Fenoll et al., 2002]. In plants phenol oxidases play an important role in biosynthesis of phenolics, and defensive role in case of cell damages and/or penetration of foreign compounds (xenobiotics) in plants cell. The enzymes are responsible also for browning in plants [Marshall et al., 2000]. There are quite a few publications and special reports concerning tea leaf phenol oxidases but not much information is available about walnut green husk phenol oxidases [Pruidze et al., 2003; Omiadze et al., 2000].

The aim of this paper is to study the changes in activities of these enzymes during vegetation period and characterize comparatively tea leaf and walnut green husk phenol oxidases.

Materials and Methods

Leaves of Georgian variety of tea plant *(Camellia Sinensis L.)* „Kolkhida" and walnut green husk *(Juglans regia L.)* were field collected and used as the material for research.

Isolation of phenol oxidase from tea leaves was carried out according to the methods described in reference [Mchedlisvili, 2005].

Isolation of phenol oxidase from walnut green husk was carried out according to the methods described in reference [Zamtaradze, 2003].

Protein content in enzyme preparation was determined by Amido Black reagent [Plum, 1955].

The catechol oxidase activity was determined spectrophotometrically according to Lanzarini, *et al*. [Lanzarini et al., 1972]. One unit of enzyme activity was defined as the amount of the enzyme sufficient to change the absorption spectrum at 420-430 nm by the value 0.05 in 1 min. Specific activity was expressed in units per mg protein.

The monophenolase activity was determined from the p-cresol oxidation in the presence of proline [Drawert, Gebbling, 1963]. Reaction mixture containing the enzyme solution, 1% p-cresol, and 0.2% proline was incubated for 24 h. The optical density of the reaction mixture was measured at 530 nm with a SF-26 spectrophotometer.

Results and Discussion

Dynamics of catechol oxidase and monophenolase activities of phenol oxidases of walnut green husk during its fruit growth and vegetation period of tea plant were studied. In June, both catechol oxidase and monophenolase activities of walnut green husk phenol oxidase increased. In July the enzyme revealed maximum activity. From the middle of August monophenolase activity decreased sharply while catechol oxidase activity reduced gradually (Fig. 1). During the vegetation period of tea plant (May-October), in the leaves monophenolase activity increased gradually and reached maximum value in July-August. From September monophenolase activity gradually decreased and at the end of vegetation period this activity was not observed at all. As to tea leaf catechol oxidase activity, it increased from May and reached maximum value in August. From September catechol oxidase activity began to decrease and in October 65% of maximal activity was observed. Monophenolase and catechol oxidase activities in the plants were found to be higher in July-August, most probably due to higher metabolic activity during this development stage. The increase of monophenolase and catechol oxidase activities in the plants at this period of development indicates the intensification of biochemical processes and involvement of oxidative enzymes in these processes.

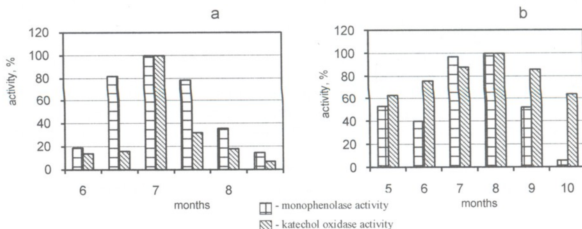

Fig. 1. Dynamics of accumulation of monophenolase and catechol oxidase in walnut green husk (during fruit formation)(a) and in tea leaf (during plant vegetation period) (b).

Both tea leaf and walnut green husk phenol oxidases revealed maximum activity at neutral pH. As shown on Fig.2, pH optimum of tea leaf monophenolase was found to be 7.1 and for walnut green husk it was pH 6.9 (p-cresol as a substrate). Monophenolase activity of tea leaf decreased sharply at pH above 7, while walnut green husk monophenolase activity decreased

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relatively slowly. In spite of some slight differences in pH dependence, both monophenolases exhibited maximum activities at pH around 7.0, which is typical for the majority of plant oxidases. pH Optima of tea leaf and walnut green husk catechol oxidases were observed at lower pH as compared with monophenolase activity.

Study of dependence of the tea leaf and walnut green husk monophenolase activities on the protein concentration indicates that at lower protein concentration the enzyme had higher specific activity (Fig.3). The increased protein concentration has a less specific activity of monophenolase. Obviously it should be taken into consideration that at the high protein concentration the probability of formation of associated enzymes forms is increased [Volkenshtain, 1981].

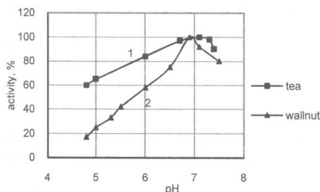

Fig. 2. Effect of pH on monophenolase activity: 1- Tea leaf monophenolase; 2- Walnut green husk monophenolase

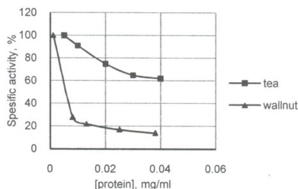

Fig. 3. Dependence of tea leaf and walnut green husk monophenolases specific activities on the protein concentration

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Dependence of tea leaf and walnut green husk monophenolases activities on the substrate (p-cresol) concentration was studied. For walnut green husk phenol oxidase dependence of the initial rate of hydroxylating reaction on the substrate concentration had a sigmoidal form. The Hill coefficient -n_H- value, estimated by plotting 1/V against lg [S], was equal to 0.2, indicating the negative cooperative kinetics [Cornish-Bowden, 1979]. The reaction kinetics did not follow Michaelis-Menten equation. Tea leaf monophenolase activity did not follow classical kinetics either. This result suggests the existence of non-equivalent active centers in the enzyme molecules or likelihood of heterogeneity of the partly purified enzymes.

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ჩაის ფოთლისა და კაკლის წენგოს ფენოლოქსიდაზების შედარებითი დახასიათება

ომიაძე ნ., მჭედლიშვილი ნ., გულუა ლ., ზამთარაძე რ., აბუთიძე მ., წიკლაური გ.

დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, 0159, თბილისი,

(მიღებულია 06.08.2007)

რეზიუმე

ჩაის *(Camellia Sinensis L.)* ფოთლიდან და კაკლის *(Juglans regia L.)* წენგოდან გამოყოფილია ფენოლოქსიდაზები. შესწავლილია ამ ფერმენტების მონოფენოლაზური (მონოფენოლ მონოოქსიგენაზა, ფ.კ.. 1.14.18.1) და კატექოლ-ოქსიდაზური (CO; ფ.კ.. 1.10. 3.1) აქტივობები. ნაჩვენებია, რომ ორივე მცენარის ფენოლოქსიდაზას აქტივობა მაქსიმუმს აღწევს ივლისსა და აგვისტოში. ჩაის ფოთლისა და კაკლის წენგოს მონოფენოლაზები ამჟღავნებენ მაქსიმალურ აქტივობას pH 7.1-სა და 6.9-ზე შესაბამისად. მონოფენოლაზური რეაქციების კინეტიკა არ ემორჩილება მიხაელის-მენტენის განტოლებას.

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Biochemistry

MOLECULAR MECHANISMS OF Mg^{++}-INDEPENDENT HCO_3^--ATPase

TSAKADZE L., DZNELADZE S., KOMETIANI Z.

Beritashvili Institute of Physiology

(Received July 23, 2007)

Abstract

Mg^{++}-independent HCO_3^- activated ATPase (HCO_3^--ATPase) was studied in brain membrane fractions. Using the method of analysis of kinetic curve of geometric shape a minimal model of HCO_3^--ATPase molecular mechanism, kinetic scheme and analytical formula of enzyme velocity have been established.

Key words: synaptosome, Mg^{++}-independent HCO_3^--ATPase

Introduction

Enzyme systems (anion ATPases) depending on anions, which accomplish ATP hydrolysis are known in the literature. Among them is Mg^{++}-dependent ATPase activated by HCO_3^- ions (HCO_3^--ATPase, E.C.3.6.1.3) [Tsakadze, Koshoridze, 1976; Ivashenko, 1977]. HCO_3^--ATPase appears to be quite distributed enzyme and is found in plasmic membrane of animals and plants. The participation of this enzyme in the process of active transport of bicarbonate ion in the membrane is supposed [Ivashenko, 1982]. But the ATPase system accomplishing ATP hydrolysis without Mg ions (Mg^{++}-independent HCO_3^--ATPase) has been observed in synaptic membrane of a brain [Tsakadze et al., 2007].

On experimental evidence the aim of our work was to develop the kinetic model of Mg^{++}-independent HCO_3^--ATPase system using the method of analysis of geometric form of kinetic curves [Kometiani, 1982].

Materials and Methods

Experimental work was carried on the fractions of albino rats' brain synaptic membranes and other membrane fractions of both sexes, weighing 200-300 g. To obtain membrane fractions and determine ATPase activity we employed the earlier described methods [Kometiani et al., 1984].

Results and Discussions

Our previous experimental researches [Tsakadze at al., 2007] enable us to conclude that in the cerebral membrane fractions (microsomes, synaptosomal membranes, mitochondria) proceeds

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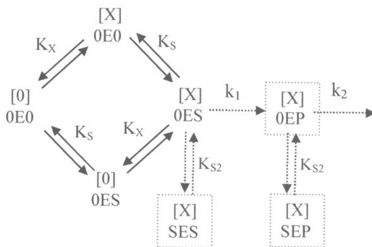
Mg^{++}-independent HCO_3^--stimulated ATP hydrolysis. Free ATP is the substrate for HCO_3^--ATPase, while HCO_3^--ions are the activators of enzyme reaction. The variation of substrate concentration plays a crucial role in ascertaining the molecular mechanism of the enzyme. For a low concentration of substrate (S<1 mM) the reaction velocity 1/V=f(1/S) dependence is rectilinear, within high (S>1 mM) concentration domain 1/V=f(S) it is not rectilinear. HCO_3^--ATPase functional unit has two substrate binding sites – one is catalytic, the other – inhibitory. This is perhaps indicative of its being a dimmer like P-type transport ATPase. It is clear that HCO_3^- - is a mixed modifier (activator) of constant affinity for the substrate.

On the basis of the above-said evidence and theoretical calculations one may fancy a principal (kinetic) scheme for the operation of HCO_3^- -ATPase: (so-called minimal model which implies selection of enzyme forms and a minimal number of reaction steps between them, which are bound observing a definite rule and at any concentration of a ligand provide coincidence of geometric configurations of theoretical and experimental curves [Kometiani, 2005]):

Scheme



In the scheme S is ATP, X – HCO_3^-- ions, P_i – inorganic phosphate. OEO designates the enzyme form, when not a single nucleotide binding site is occupied (E implies functional unit of the enzyme); OES is the catalytic site of substrate-bound form of enzyme (S<1 mM) to which modifier (X) binds; OEP is the phosphorylated intermediate of the enzyme. In the scheme, depending on the type of ATPase we have to deal with V- or P-type. At high substrate concentration two versions of enzyme line-end branching form can be possible: 1 SES, when an enzyme molecule binds with another S only in the case when it has already been bound with X and the first S (V-type ATPase); 2) SEP, which represents the phosphorylated intermediate, to which only in this condition binds another ATP (P-type ATPase). SES and SEP forms of enzyme represent **dead-end branchings**.

As a result of theoretical analysis of V=f(S, X) dependence and its comparison with experimental curves, in terms of the type (V or P) of HCO_3^--ATPase, analytical formula of velocity will assume the following pattern:

$$V = \frac{e_0 k \frac{XS}{K_X K_S}}{\left(1+\frac{X}{K_X}\right)\left(1+\frac{s}{K_S}\right)+\frac{XS^2}{K_X K_S K_{S2}}}$$ V-type ATPase

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$$V = \frac{e_0 k_1 k_2 \dfrac{XS}{K_X K_S}}{k_2\left(1+\dfrac{X}{K_S}\right)\left(1+\dfrac{S}{K_S}\right)+k_1\dfrac{XS^2}{K_X K_S K_{S2}}}$$ P-type ATPase

where, V is the reaction velocity, k_1 and k_2 velocity catalytic constants, while K_x, K_s and K_{s2} are respective apparent dissociation constants.

P-type ATPase – has phosphorylated intermediate.

V-type ATPase – has not phosphorylated intermediate.

Thus, on the basis of experimental evidence the molecular mechanism of synaptic membrane Mg^{++}-independent HCO_3^--ATPase has been studied. Development of Mg^{++}-independent HCO_3^--ATPase kinetic model will enable to ascertain physiological function of anionic ATPases, which is so far undetermined. Their place in the ATPase general classification is also to be specified.

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Tsakadze L., Dzneladze S., Kometiani Z. Proc. *Mg^{++}-independent HCO_3^--ATPase.* Georgian Acad. Sci., Biol. Ser. B. **5**, 2, 9-13, 2007.

Mg^{++}- არადამოკიდებული HCO_3^--ATPაზას მოლეკულური მექანიზმი

წაქაძე ლ., ძნელაძე ს., ქომეთიანი ზ.

ივ. ბერიტაშვილის სახელობის ფიზიოლოგიის ინსტიტუტი

(მიღებულია 23.07.2007)

რეზიუმე

თავის ტვინის მემბრანულ ფრაქციებში გამოკვლეულია Mg^{++}-არადამოკიდებული HCO_3^--აქტივირებული ATPაზა (HCO_3^--ATPაზა). კინეტიკური მრუდის გეომეტრიული ფორმის ანალიზის მეთოდის გამოყენებით დადგენილია სინაფსური მემბრანის HCO_3^--ATPაზას მოლეკულური მექანიზმის მინიმალური მოდელი, კინეტიკური სქემა და ფერმენტული სიჩქარის ანალიტიკური ფორმულა.

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Biophysics

INTERRELATION OF NATIVE ACTOMYOSIN ACTIVE AND ACTIN-BINDING CENTRES

GOGORISHVILI J., GACHECHILADZE N., TORIASHVILI T., ZAALISHVILI T., ZAALISHVILI M.

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Abstract

The influence of iodoacetamide (IAA) on rabbit skeletal muscle native actomyosin has been studied using viscosimetry, superprecipitation, fermentative and calorimetric methods. It has been shown that myosin actin centre that binds with actin at any ionic strength and actin centre that creates additional bonds are ruled by different SH-groups. According to calorimetric data denaturational curve of IAA-treated natural actomyosin is analogical to that of the system - actomyosin + Mg^{2+} -ATP. The transition temperatures and enthalpies of IAA - treated and nonmodified native actomyosins are 42.5°C and 1.7 cal/g and 45.7°C and 4.7 cal/g correspondingly.

Key words: Iodoacetamide, viscosity, superprecipitation

Introduction

The study of interrelation of native actomyosin active and actine-binding centres is still actual. Actin centres mean both the centre that binds with actin at any ionic strength and the centre that for this binding needs intermediative complex - x P_i ADP, where x is myosin active centre [Poglazov and Levitski, 1982].

Myosin active centre was shown to be practically inhibited under the high concentrations of iodoacetamide (IAA), but the centre, that binds with actin at any ionic strength, was not bound [Barany M. and Barany K., 1959; Perry,1967]. Naturally, the question arises – what happens at this time with myosin actin-binding centre that creates the additional bonds with actin. This task has not been investigated earlier [Barany M. and Barany K., 1959].

According to Takeuchi K and Tonomura V (1971) two subfragment 1-s of myosin are not identic. While acting with ATP both acto-subfragment 1-s dissociate, but one of them splits ATP and the other does not, i.e. 1 mole of myosin splits 1 mole of ATP. How these data obtained by light scattering method correspond to the generally acknowledged fact that under the influence of Mg^{2+} ions and Na-pyrophasphate actomyosin dissociates into actin and myosin. This question needs more precise definition.

This article presents the results of our investigation carried out on untreated and IAA-modified native actomyosin of rabbit skeletal muscle. The main task of our study was to determine how much actin centre binding with actin at any ionic strength was independent from myosin active centre. In the case if it was not fully independent we wanted to determine whether the same SH-

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groups (IAA is -SH reagent) rule the dissociation of actomyosin complex and creation of additional bond between actin and myosin, i.e. superprecipitation, or no.

Materials and Methods

The native actomyosin was obtained from rabbit skeletal muscle using Weber-Edsale solution (0.6 M KCl; 0.04 M $NaHCO_3$; 0.01 M Na_2CO_3). The natural actomyosin was extracted from diminished muscle by 24 h unbroken stirring at 0°C. The quantity of added extraction buffer was 5 volumes. The extract was either directly filtered in linen sack and then centrifugated at high speed, or first diluted by the same volume of 0.6 M KCl and then centrifugated. The upper solution was diluted by 15 volumes of icy distilled water, precipitated actomyosin was gathered by centrifugation and then precipitated once or twice. The native actomyosin was also obtained according to Szent-Gyorgyi using Guba-Shtraub buffer (0.6 M KCl; 0.1 M KH_2PO_4; 0.05M K_2HPO_4; pH 6.5 -7.0) [Gogorishvili, 1998].

The native actomyosin was treated by IAA during 2 hours at room temperature, pH 8 (0.1 M IAA; 0.02 M Tris-HCl, proten concentration 5-10 mg/ml) [Barany M and Barany K, 1959]. After the treatment it was precipitated 3 times. The protein viscosity was determined by Ostwald viscosimeter (the time of pure solvent flowing out - 30sec).

The ATP sensitivity was estimated by formula:

$(\log \eta_r - \log \eta_{rATP} / \log \eta_{rATP}) \times 100$,

where $\log \eta_r$ –are actomyosin relative viscosities (treated by IAA or control) before ATP addition and $\log \eta_{rATP}$ - actomyosin viscosities (treated by IAA or control) after the addition of 10^{-3} M ATP.

The protein concentration was determined by biuret method [Gornall et al., 1949] and its ATP activity – according to splitted terminal phosphate. Inorganic P was defined by extraction method using isobutanol [Turakulov et al., 1967].

The ability to superprecipitation of native AM was established according to increase of its optical density at λ=550nm wavelength.

Calorimetric measurements of IAA-treated and nonmodified natural actomyosins were taken using the differential calorimeter [Monaselidze et al., 2006].

Results and Discussion.

Our first experiments showed that at high ionic strength (0.5 M KCl) pH 9 IAA (0.1 M) did not fully inhibit ATP-ase activity of native actimyosin though decreased it about 10 times (Table 1). It was interesting to clarify how did the viscosity of IAA-treated protein decrease in those conditions after the adding of ATP.

Table 1. ATP-ase activity of nonmodified and IAA-treated native actomyosin

pH	Ionic strength I	ΔP_i		Added ions
		Nonmodfied	Modfied	
9.0	0.5	0.050	0.004	10^{-2}M Ca^{2+}
7.0	0.1	0.130	0.120	2.5 10^{-3}M Mg^{2+}

ΔP_i - ATP-ase activity (splitted P_i/mg.protein min); ATP concentration - 5. 10^{-3} M; Buffer - 0,02 M Tris-HCl T=30°C

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Table 2. ATP sensitivity of nonmodified and IAA-treated natural actomyosin

Native actomyosin	η_r	η_{rATP}	ATP-sensitivity (%)	pH
Nonmodified native actomyosin	2.76	1.26	340	9.0
IAA-treated native actomyosin	2.30	1.70	40	9.0
Nonmodified native actomyosin	2.23	1.26	247	7.0
IAA-treated native actomyosin	———	———	———	7.0

Buffer - 0.02M Tris-HCl, 0.5M KCl; ATP was added up to 10^{-3}M final concentration; T=20°C

Table 2 shows that ATP sensitivity is also decreased about 10 times. Hence, myosin actin centre that binds to actin at any ionic strength is not fully independent from active centre. It must be mentioned that at high ionic strength (pH 7.0) the adding of ATP to IAA-treated native actomyosin causes the precipitation of protein and that is why the corresponding line in Table 2 is blank. One can conclude that myosin active centre has strategically such important position that it influences the mentioned actin centre. Calorimetric investigations also indicate to the strategic importance of active centres (see below).

In physiological conditions (pH 7.0; ionic strength I=0.1) the actin centre, that for binding with actin needs intermediative complex - x P_i ADP, is comparatively independent from active centre (Table 1). This comparative independence of actin centre from modificator can be explained only by the difference of SH-groups ruling myosin actin centre that binds to actin at any ionic strength from SH-groups ruling the creation of additional bonds (we mean steric factors). The superprecipitation of IAA-treated and nonmodified native actomyosin witnesses the same: the change of optical density per 1mg of protein is 0.050 for nonmodified and - 0.042 for IAA- treated native actomyosin.

The fact that myosin actin centre that binds to actin at any ionic strength depends on active centre agrees with experiments carried out by means of natriumpyrophosphate and Mg^{2+} ions. After the modification of active centre by IAA the adding of natriumpyrophosphate ($5\cdot10^{-3}$M) and Mg^{2+} ($5\cdot10^{-3}$M) changes the viscosity in the same way as ATP (it decreases about 10 times comparing with that of nonmodified one). In the case of nonmodified native actomyosin the functioning of ATP and Mg^{2+}-pyrophosphate is adequate from the view of viscosity decrease. Naturally, the revealing of dependence of mentioned actin centre on myosin active centre needs the presence of IAA. IAA has clarified this question.

Basing on the Takeuchi et al. (1971) data we see the problem in the following way: acto-subfragment 1 and acto-heavy meromyosin (HMM) under the ATP influence dissociate at any ionic strength, and actomyosin - under the ATP, as well as Mg^{2+}-pyrophosphate influence - only at high ionic strength. Myosin keeps its native structure better than subfragments isolated from it; e.g. ATP-ase activity of HMM is activated by Mg^{2+}, while ATP-ase of myosin is inhibited [Perry, 1967] and this fact has a great physiological importance for muscle being in rest condition from the view of economical spending of ATP. Besides, neither subfragment 1, nor HMM creates additional bond with actin by means of ATP. Despite of abovementioned the study of Mg^{2+}-pyrophosphate influence on even only HMM is still desirable. The aim of such study is to clarify whether only ATP causes the dissociation of acto-HMM (as well as of acto-subfragment 1) or no and what accompanies myosin splitting into subfragments.

We couldn't manage to fully inhibit ATP-ase activity of native actomyosin (pH -9.0; I-0.5; Table 1) using the high concentrations of IAA (0.06 M; 0.08 M; 0.10 M). As we have already mentioned the little decrease of viscosity corresponds to low ATP activity (Table 2), but

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when after ATP addition we bring pyrophosphate and Mg^{2+} into reaction area, we obtained paradoxic result – the viscosity rose up to initial value. This fact is still unexplained, though one can say that in this case inorganic pyrophosphate and ATP are rivals.

The viscosity of nonmodified native actomyosin having been decreased under the ATP influence restores simultaneously with the bringing of 10^{-2}M Ca^{2+} into the reaction area, while the viscosity of IAA-treated one – does not. This fact can be explaned in a way that Ca^{2+} increases ATP-ase activities of myosin and actomyosin, though in the case of IAA-treating Ca^{2+} cannot reveal its ability.

Fig. 1 and Fig. 2 show calorimetric data of nonmodified and IAA-treated native actomyosins. One can see that treating by IAA sharply changes the thermogram of natural actomyosin denaturation: the transition temperature of nonmodified actomyosin is 45.7°C while that of treated one – 42.5°C. Correspondingly, the enthalpy (transition heat) of nonmodified actomyosin is 4.7 cal/g and of IAA-treated - 1.7 cal/g. But the most important is that denaturation thermogram of modified actomyosin is analogical with that of system - skeletal muscle actomyosin + Mg^{2+}-ATP (Fig. 3). It confirms that, first, IAA really binds (constrains) myosin active centre and, the second, SH-groups directly participate in the interaction with ATP. Besides, active centre has strategically very important position in myosin.

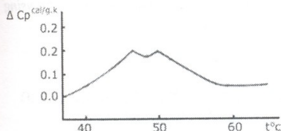

Fig.1. Thermogram of nonmodified native actomyosin denaturation. Protein concentration - 26.0 mg/ml; I - 0.5; pH - 7.0

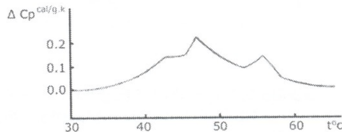

Fig.2. Thermogram of IAA-treated native actomyosin denaturation. Protein concentration - 22.5 mg/ml; I - 0.5; pH - 7.0

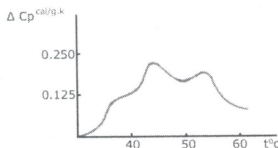

Fig.3. Thermogram of denaturation of system actomyosin+ Mg^{2+} -ATP. Protein concentration -6.3 mg/ml; 5.10^{-4}M Mg^{2+}-ATP; I - 0.5; pH - 7.0

To exclude the influence of tropomyosin, troponin and titin further we are going to study reconstructed actomyosin by means of the same methods.

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ბუნებრივი აქტომიოზინის აქტიური ცენტრისა და აქტინური ცენტრების ურთიერთკავშირის შესწავლისათვის

გოგორიშვილი ჯ., გაჩეჩილაძე ნ., ტორიაშვილი თ., ზაალიშვილი თ., ზაალიშვილი მ.

მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია16.07.2007)

რეზიუმე

ვისკოზიმეტრიის, ფერმენტული, სუპერპრეციპიტაციისა და კალორიმეტრული მეთოდების გამოყენებით შესწავლილია SH-ჯგუფების რეაგენტის - იოდაცეტამიდის გავლენა ბოცვერის ჩონჩხის კუნთების ბუნებრივი აქტომიოზინის ფიზიკურ-ქიმიურ თვისებებზე. დადგენილია, რომ მიოზინის იმ აქტინურ ცენტრს, რომელიც კავშირს ამყარებს აქტინთან ნებისმიერი იონური ძალის პირობებში და იმ აქტინურ ცენტრს, რომელიც დამატებით კავშირს წარმოქმნის აქტინთან, აქტიური ცენტრის სხვადასხვა SH-ჯგუფები მართავენ. კალორიმეტრული გამოკვლევების თანახმად იოდაცეტამიდით დამუშავებული ბუნებრივი აქტომიოზინის დენატურაციის მრუდი ანალოგიურია სისტემის - აქტომიოზინი + Mg^{2+}-ATP დენატურაციის მრუდისა. იოდაცეტამიდით დამუშავებული და დაუმუშავებელი აქტომიოზინის გადასვლის ტემპერატურა და გადასვლის ენტალპია შესაბამისად არის 42.5°C და 1.7 კალ/გ და 45.7°C და 4.7 კალ/გ.

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THE MORPHOLOGICAL STUDY OF ACTOMYOSIN AND α-ACTININ-ACTOMYOSIN COMPLEX IN THE PRESENCE OF UNIVALENT IONS

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Abstract

The structural changes of actomyosin and α-actinin–actomyosin complex in the presence of univalent ions have been studied by means of electron microscopical method. It was established that in the presence of some univalent ions the actomyosin structure changed that caused the changing of velocities of ATP-ase reaction and superprecipitation, and α-actinin independently of the actomyosin structure changes initiates the association of actomyosin bundles.

Key-words: α-actinin, actomyosin, actomyosin bundles.

Introduction

It is known that a lot of ferments, which catalyze different reactions in living organisms, are activated by ions. Muscle proteins - myosin and actomyosin are such proteins and for their binding with ATP and pronouncing of ATP-ase activity the presence of divalent (Mg^{2+}, Ca^{2+}), as well as univalent ions, is necessary, as they give definite charge and configuration to contractile system [Bandoll, 1979; Zaalishvili, 1971].

It is established that the contractile systems of muscle and nonmuscle cells include a great number of minor proteins, which participate in the contractive processes. One of such proteins is α-actinin. The presence of this protein in many organs and tissues points to the fact that it must carry out an important function. Therefore, to learn functional role of α-actinin in biological motion the study of its influence on the properties of actomyosin systems at different conditions is necessary.

Previously we established that univalent ions (cations, anions) affected the velocities of ATP-ase and superprecipitation reactions of an actomyosin and α-actinin - actomyosin complex [Pavliashvili et al., 1982]. The influence of univalent ions on the line of "efficiency" is distributed as follows: $Na^+ \geq K^+ \geq Rb^+ > Li^+ \geq Cs^+$ for cations, and $Cl^- > F^- > Br^- > I^-$ - for anions. The changes of temperature, pH and ionic strength do not change the ions order on the line of "efficiency", and effect of α-actinin on actomyosin properties is the same. It is necessary to note that in the presence of anions the evident influence of α-actinin on the properties of actomyosin is observed, e.g. in the presence of Br^- ions the adding of α-actinin increases velocity of actomyosin superprecipitation, while actomyosin itself in the presence of Br^- doesn't superprecipite [Pavliashvili, 1984].

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The purpose of the presented work was the study of the effect of some univalent ions on the structure of α-actinin-actomyosin complex by electron microscopical method.

Materials and Methods

All proteins were obtained from rabbit skeletal muscle. Actin was obtained according to the method of Rees and Yong (1967); myosin - according to the method of Perry [Perry, 1958]; the α-actinin - by the method of Pinter [Pinter, 1980]; the reconstructed actomyosin was obtained by mixing myosin and F-actin in ratio 3:1 (by mass) in 0.4-0.5 M KCl; the complex of α-actinin-actomyosin was obtained by mixing of α-actinin with actomyosin. The concentration of the protein solutions were measured by means of Burette reagent [Beily, 1965]; for electron microscopical study myosin, actomyosin, actin, α-actinin preparations were prepared in the following way: the patterns with concentration 0.5 mg/ml were precipitated on the formvar film and contrasted by 1% uranilacetate water solution. We used the electron microscope – 100-AK, 75 kV, ×44000.

Results and Discussions

At the first step of the work the structures of F-actin and myosin threads in the presence of Lithium and Cesium (LiCl and CsCl, with concentration 0.1M; pH 7.5; T=0°C) have been studied. Their structures were accordingly compared with the structures in 0.1 M KCl that had been studied beforehand [Kupatadze et al., 2006].

Presented electrophoregrams show that in the presence of Cs^+ ions F-actin bundles partly dissociate into threads, lose rigidness, band and shorten, and thick myosin threads (7-10 μm) became thinner (2–5μm) (Fig. 1 a,b). Those structures in the presence of Li^+ under the similar conditions remain the same as with KCl. One can see the isolated as well as aggregated side-to-side threads of F-actin and myosin threads that have characteristic structure, the bipolarity of threads are distinctly seen, the heads disposed on the surface of the threads with the periodicity 14.3 μm [Pavliashvili, 1982].

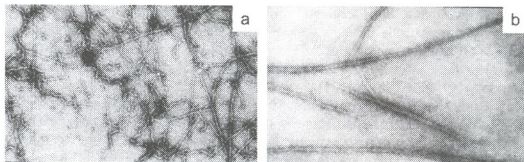

Fig. 1. a - F-actin threads; **b** - Myosin threads in 0.1 M CsCl, pH 7.5, T=0°C

In spite of the fact that in the presence of Li^+ and Cs^+ ions the structures of F-actin and myosin threads differ from each other, the structures of actomyosin reconstructed from them are similar, however in actomyosin bundles the thick threads of myosin are thinner than in the presence of K^+ ions (Fig. 2 a,b). The electromicroscopical research of α-actinin-actomyosin complex showed that in the presence of K^+ ions under the influence of α-actinin actomyosin was presented as tight bundles. On the electrophoregram separately disposed threads of actin were evidently seen [Kupatadze, 2006]. The corresponding electrophoregrams in the presence of Cs^+ and Li^+ ions differ

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from each other, though α-actinin is still capable to create actomyosin bundles but relatively less tight (Fig.2 c,d).

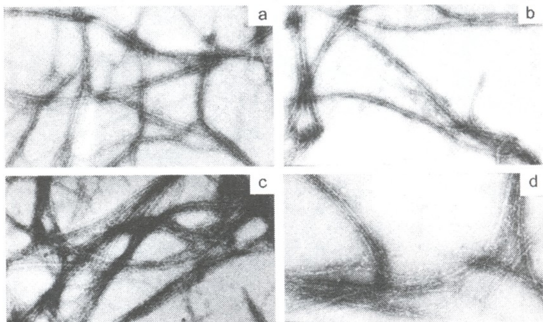

Fig.2. a - actomyosin structure in 0.1 M CsCl; **b** - actomyosin structure in 0.1 M LiCl; **c** - structure of α-actinin–actomyosin complex in 0.1 M CsCl; **d** - structure of α-actinin–actomyosin complex in 0.1 M LiCl; pH 7.5, T=0°C

Proceeding from above mentioned we can conclude that the substitution of one univalent cation into another in the reaction area causes the definite changes in the structure of proteins as well as in the structure of their complexes.

Then we studied thin threads of F-actin and myosin in 0.1M KF (pH 7.5, T=0°C). Fig. 3 a,b show that isolated as well as aggregated side-to-side threads of F-actin with the length of 13.3 μm, and the threads of myosin that have characteristic structure: the bipolarity of threads is distinctly seen, their structures slightly differ from the structures of actin and myosin threads in 0.1M KCl.

On the electrophoregram of reconstructed actomyosin the rotation of thin (F-actin) and thick (myosin) threads, as well as actomyosin bridges are distinctly seen (Fig. 3 c,d). In these conditions the structure of myosin is regulated but less than in KCl [Kupatadze et al., 2006].

The adding of the third component - α-actinin causes the changes in the structure of actomyosin complex. Under the influence of α-actinin actomyosin complex assembles into the tight bundles around which no free actin threads occur, that is observed in the case of actomyosin.

As for F-actin and myosin threads in the presence of I^- ions (0.1MKI; pH 7.5, T=0°C) the structure of F-actin is destroyed and molecules of myosin are assembled into shorter (0.5 μm) and thinner (4-5 μm) threads (Fig. 4 a,b). Actomyosin, reconstructed from the F-actin and myosin threads is less regulated system. On the background the thin aggregates of myosin are seen (Fig. 4c). When α-actinin is added to such actomyosin the loosening of actomyosin bundles occurs, but the thin aggregates of myosin is still seen on the background (Fig. 4d). It is interesting that such actomyosin has ATP-ase activity, but it does not superprecipite.

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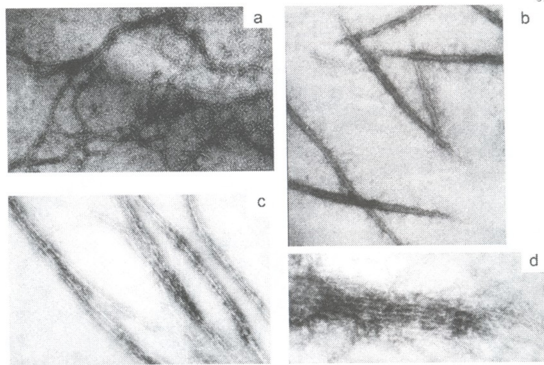

Fig. 3. a - F-actin threads; **b** - myosin threads; **c** - structure of actomyosin; **d** - structure of α-actinin–actomyosin complex; in 0.1 M KF, pH 7.5, T=0°C

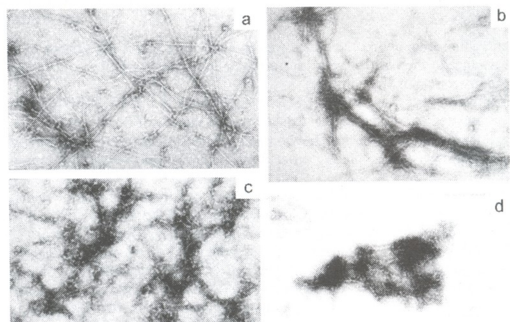

Fig.4. a - F- actin threads; **b** - myosin threads; **c** - structure of actomyosin; **d** - structure of α-actinin–actomyosin complex; in 0.1 M KI, pH 7.5, T=0°C.

Thus the received results show that in the presence of some univalent ions the structure of actomyosin is changed. As a result the velocities of ATP-ase reaction and superprecipitation are changed, and independently of the actomyosin structure α-actinin is able to produce actomyosin bundles.

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აქტომიოზინისა და α–აქტინინ-აქტომიოზინის კომპლექსის მორფოლოგიური კვლევა ერთვალენტიანი იონების თანაობისას

კუპატაძე რ., სიმონიძე მ., ქურიძე კ., ჯაფარიძე ი., დოლიძე მ., ერისთავი თ., ზაალიშვილი მ.

მოლეკულური ბიოლოგიისა და ბიოლოგიური ფიზიკის ინსტიტუტი

(მიღებულია 09.07.2007)

რეზიუმე

შესწავლილია აქტომიოზინისა და α-აქტინინ-აქტომიოზინის კომპლექსის სტრუქტურული ცვლილებები ერთვალენტიანი იონების თანაობისას ელექტრონულმიკროსკოპული მეთოდის გამოყენებით. დადგენილია, რომ ზოგიერთი ერთვალენტიანი იონის თანაობისას იცვლება აქტომიოზინის სტრუქტურა, რის შედეგადაც იცვლება ATP-აზური და სუპერპრეციპიტაციის რეაქციათა სიჩქარეები და დამოუკიდებლად აქტომიოზინის სტრუქტურისა α-აქტინინს უნარი შესწევს წარმოქმნას აქტომიოზის კონები.

საქართველოს
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IDENTIFICATION OF THE MEDICINAL PLANTS CITED IN KARABADINES (BOOKS OF OLD GEORGIAN MEDICINE AND PHARMACY)

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Abstract

A new original method for the identification of the herbal components of remedies mentioned in ancient Georgian medical books – Karabadines is described. Old medical sources of different countries were compared with Georgian Karabadines. The analogous recipes were found and by comparing their components etymology of plant names has been established. List of 7 medicinal plants identified by this method is given: "Thiva Katsis Dziri" (*Mandragora officinalis* L.), "Abkia" (*Mentha pulegium* L.), "Adkhari, Puka Alathkhiri" (*Andropogon schoenanthus* L.), "Moi" (*Valeriana officinalis* L. et *Valeriana phu* L.), "Poi" (*Meum athamanticum* Jacq.), "Damotis Tesli", "Bulgri" (*Laurus nobilis* L.), "Sumbuli Tibi" (Sumbul from Tibet).

Key words: Georgian folks medicine, Karabadini, medicinal history, medicinal plants, medicinal recipes, pharmacy.

Introduction

Decoding of the medical components mentioned in ancient Georgian medical books, in Karabadines [Qananeli, 1940; Khoja Kopili, 1936; Zaza Panaskertel-Tsitsishvili, 1978; D. Batonishvili, 1985], is of great theoretical and practical value with the purpose of search for new medicines for modern pharmacy. The herbs are major components and often the basis for modern pharmacological preparations.

In 20th century a lot of researches were conducted for identification of these plants and their names and various dictionaries ware compiled [Bagrationi, 1986; Annenkov, 1878; Kotetishvili, 1940; Shengelia, 1978; Janelidze 2002].

Despite of successes in this field, there are many medical terms, which are not identified till now.

For decoding the old names of those medical plants we offered a new method of comparison of Arabian recipes with Georgian ones. On the basis of such comparison plants, which were applied in the Middle Ages in Georgian pharmacy have been revealed.

Materials and Methods

The data of comparison of the recipes taken from the different literature sources are given in the table. The list of Georgian recipes with their botanic names (in their exact order) is given in

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the first column; in the second column there is exact list of Ibn Sina's recipes with definitions; the results and explanations are given in the third column.

Table. Comparison of medicinal components in analogous recipes by Zaza Panaskerteli-Tsitsishvili's Karabadini and Ibn Sina.

Zaza Panaskerteli-Tsitsishvili, (p.191) – "Akrasal qvaqabda" (in Georgian)	Ibn – Sina, (vol 5, p.127) "Star - Patty … Kavkaba" (in Russian)	Comparisons and explanations
I) "Muri" *(Commiphora myrrha* Eng.*)*	1) "Mirra" *(Commiphora myrrha* Eng.*)*	I) "Muri" 1) "Mirra" (*Commiphora myrrha* Eng.)
II) "Takhuis Kueri" *(Castoreum)*	2) "Bobrovaia Struia" *(Castoreum)*	II) "Takhuis Kueri" 2) "Bobrovaia Struia" *Castoreum - the glandular secretion of the Castor fiber*
III) "Sumbuli Tibi"	3) "Sumbul"	III) "Sumbuli Tibi" 3) "Sumbul" *Ferula sumbul*, (Kauffm.) Hook. f. seu *Nardostachys jatamansi* D.C. seu *Hyacinthus* spp.
IV) "Tikha Berdznuli" -*printed Greece clay*	4) Ceylon's Cinnamon (*Cinnamomum cassia* (L.) C. Presl seu *C. zeylanicum* Blume*)*.	IV) "Tikha Berdznuli"- 5) printed Greece clay – printed clay
V) "Salikha" (*Cinnamomum cassia* L. seu *C. zeylanicum* Blume*)*	5) Printed clay	V) "Salikha" 4) Ceylon's Cinnamon *Cinnamomum cassia* L. seu *C. zeylanicum* Blume.
VI) **"Tiva katsis Dziri "** *(unknown)*	6) **Mandragora's root bark** *Mandragora officinalis* L.	VI) **"Tiva katsis Dziri "** is 6) *Mandragora officinalis* L.
VII) "Zaphrani" *(Crocus sativus* L.*)*	7) "Opii" *(Papaver somniferum* L.*)*	VII) "Zaphrani" 8) "Shafran" *Crocus sativus* L.
VIII) "Qushti" *(Saussurea lappa* (Decne.) Sch. Bip.*)*	8) "Shafran" *(Crocus sativus* L.*)*	VIII) "Qushti " 9) "Kust " *Saussurea lappa* (Decne.) Sch. Bip.
IX) "Aphioni" *(Papaver somniferum* L.*)*	9) "Kust" (*Saussurea lappa* (Decne.) Sch. Bip.)	IX) „Aphioni" 7) „Opii" - opium from *Papaver somniferum* L.

Results and Discussion

1) **"Tiva katsis Dziri"** - For identification of this component we have compared identical recipes mentioned in Karabadines and Ibn-Sina's books: Karabadini [Zaza Panaskerteli-Tsitsishvili, 1978, p.191] "Patty Akrasal Kvakabda" and "King Phiphad's drug [Zaza Panaskerteli-Tsitsishvili, 1978, p.117, p.97] with Ibn-Sina's recipe – "Star Patty Kavkaba" [Ibn Sina, 1980, p.127] and "Drug Paste of King Kumada". [Ibn Sina, 1980, p.54]. With the method of comparison of recipes given in those books we have established that "Thiva Katsis Dziri" is relevant with root bark of Mandragora. The first step of the method was to found the corresponding component by recipes. Second step was the identification of etymology of medical term. Ibn Sina compares Mandragora's root to the shape of the man [Ibn Sina, 1980, p. 266] and Amirdovlat Amasiatsi to the bush [Amirdovlat Amasiatsi, 1990, p.409-410], so we can explain the Georgian name **"Thiva Katsis Dziri"** which in translation means "a root of a hay-man".

2) **"Sumbuli Tibi"** – M. Janelidze indicates [Janelidze, 2003] that according to A. Makashvili's "Botanical Dictionary" [Makashvili, 1961] Sumbul is *Hyacinthus orientalis* L.,

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while in N. Tsutsunava's opinion Sumbuli is *Nardostachys jatamansi* D.C. or *Ferula moschata* (H. Reinsch) Koso-Pol. (*F. sumbul* (Kauffm.) Hook. f;.) [Tsutsunava, 1966].

Hyacintus belongs to the Hyacinthaceae family, while Nardostachys is ascribed to the Valerianaceae family. So they are different plants and it is impossible to regard them as the same plants.

What the word "Tibi" means is unknown till now. Based on comparison method Sumbuli Tibi was relevant with aroma sumbuli, which according to Ibn Sina (1980, §516, p.470) had the name "Sparrow Sumbul" as the synonym. Biruni notes that "Sparrow Sumbuli" grows in India, Kashmir and Tibet [Al-Biruni, 1974, §571, p.552]. Hence, we can suppose that "Tibi" denotes its homeland - Tibet.

3) "**Abkia"** - This plant is mentioned in Karabadine [Zaza Panaskerteli-Tsitsishvili, 1978, p.97] in recipes: "King Phiphad's Drug" and in "King Kumada's drug" [Zaza Panaskerteli-Tsitsishvili, 1978, p.117]. Analogue of those both recipes is Ibn-Sina's recipe "Drug Paste of King Kumada" [Ibn Sina, 1980, vol.5, p. 54]. If we compare the components of all these recipes we'll see, that Abkia is relevant with *Mentha pulegium* L. Mint's (Mentha). Arabian name is Habbak, which in Georgian transformed as the name – Habbaki-Abaki - Abki-Abkia.

4) **"Adkhari, Atkhari, Puka Alathkhiri**" - This component is explained as *Acorus calamus* L. by Georgian authors [Kotetishvili, 1940; Janelidze, 2002; Tsutsunava, 1966]. We compared recipe of "King Phiphad's Drug" [Zaza Panaskerteli-Tsitsishvili, 1978. p.97] with Ibn-Sina's recipe "King Kumada's drug" [Ibn Sina, 1980, vol.5, p.54] and we have found that Itkhari is *Andropogon schoenanthus* L., which in Arabian sounds as Itkhiri and its flower as "Fukkah", consequently, we have got the name – "Puka Alathkhiri".

With the same method we identified the following plants:

5) **"Moi da** - Moi is Valeriana officinalis L. et Valeriana phu L.,

6) " **Poi"** - Poi is *Meum athamanticum* Jacq.

7) "Damotis Tesli", "Bulgri" (*Laurus nobilis* L.)

Using the same method we have identified another 9 plants earlier [Chanturia, Eradze, 2006]:

8) **"Akirkara"** - *Anacyclus pyrethrum* (L.) Cass.

9) **"Seed of Damoti"** and **"Bulgri"** – seed of *Laurus nobilis* L.

10) **"Bache, Beachi"** – *Cydonia oblonga* Mill.

11) **"Zovpra, Zopa"** - *Seseli tortuosum* L., *Seseli transcaucasicum* (Schischk.) Pimenov & Sdobnina.

Soft Zovpra – Lanoline;

dry Zovpra – Hyssopus officinalis L.

12) **"Mukly"**- gummi of *Commiphora africana* (A. Rich.) Eng.

13) **"Skorkly"** – flake of the wooden house wall.

14) **"Gapthi, Gupthi"** – *Eupatorium cannabinum* L.

15) **"Qabri"** – *Capparis spinosa* L.

16) **"Hindakoki, Andokoki"** - is *Trigonella caerulea* (Desr. ex Lam.) Ser.

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ძველ ქართულ მედიცინასა და ფარმაციაში (კარაბადინებში) ციტირებულ სამკურნალო მცენარეთა იდენტიფიკაცია

ჭანტურია თ.

შ.პ.ს. "ქართული ტრადიციული მედიცინისა და ჰომეოპათიის ლიგა"

(მიღებულია 4.06.2007)

რეზიუმე

აღწერილია ძველ ქართულ სამედიცინო წყაროებში (კარაბადინებში) მოხსენიებულ სამკურნალო საშუალებებში შემავალი მცენარეული (და ზოგიერთი არამცენარეული) კომპონენტის იდენტიფიკაციის ახალი ორიგინალური მეთოდი და წარმოდგენილია ამ გზით დადგენილი სამკურნალო მცენარეების სია. ამისათვის გამოყენებულია სხვადასხვა ქვეყნის ძველი სამკურნალო წყაროები და ისინი შედარებულია ქართულ კარაბადინებთან. შემდგომ მოძიებულია ანალოგიური რეცეპტები და კომპონენტთა შედარების გზით და მცენარეთა სახელების ეტიმოლოგიის დადგენის შედეგად იდენტიფიცირებულია აქამდე უცნობი სამკურნალო საშუალებები: "თივა კაცის ძირი" (*Mandragora officinalis* L.), "სუმბული ტიბი" (ტიბეტიდან ჩამოტანილი სუმბული), "აბკია" (*Mentha pulegium* L.), "ფუკა ალადხირი" *(Andropogon schoenanthus* L.), "ფოი" (*Valeriana phu* L. *et V. officinalis* L.), "მოი" *(Meum athamanticum* Jacq.*)*, "აყირყარა" *(Anacyclus pyrethrum* L.*)*, "დამოთის თესლი", "ბულღრი" *(Seed of Laurus nobilis* L.); "ბაჩე", "ბეაჩი" (*Cydonia oblonga* Mill.), "ზოვფრა", "ზოფა" *(Seseli tortuosum* L., *Seseli transcaucasica* (Schischk.) Pimenov & Sdobnina*)*, რბილი ზოვფრა (Lanoline), ხმელი ზოვფრა (*Hyssopus officinalis* L.*)*, "მუკლი" (*Bdellium, gummi of Commiphora africanum* Engl.), "სკორკლი" (ხის სახლის კედლის ნაფშვნეტი); "ღაფთი", "ღუფთი" (*Eupatorium cannabinum* L.), "ქაბრი" *(Capparis spinosa* L.*)*, "ჰინდაკოკი", "ანდოკოკი" (*Trigonella caerulea* (Desr. ex Lam.) Ser.).

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MATERIALS ON SOIL ALGAE OF AJAMETI STATE RESERVE

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Abstract

81 soil algae belonging to 5 divisions, 9 classes, 20 orders, 37 families and 51 genera were revealed on the base of investigations made in Ajameti State Reserve. Their distribution patterns in soils of various forest formations are considered. 19 algae are mentioned for the first time for Georgia's soil algoflora and 10 are new to Georgia algoflora.

Key words: Oak forest, Imeretian oak formation, Georgian oak formation, zelkova-oak formation, algae.

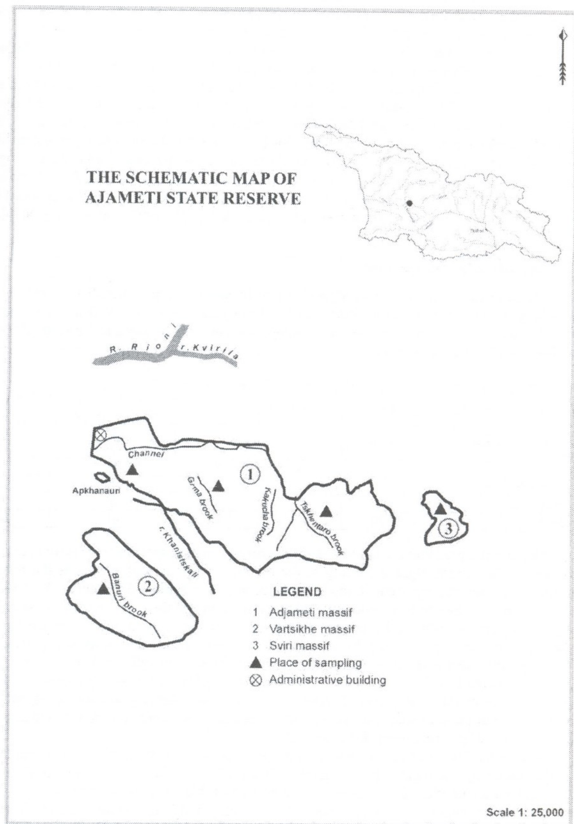
Introduction

The Adjameti State Reserve is situated in the eastern part of the Colchis lowland (West Georgia), on the valley of the river Rioni, at 150-200 m a.s.l., on territories of the village Baghdati and Zestaponi administrative district [Matsaberidze 1974]. It consists of three massifs: the Adjameti oak massif located between the rivers Kvirila and Khanistskali, left-side tributaries of the river Rioni, the Vartsikhe and Sviri oak massifs. The distance between these massifs is 2-3 km and they cover area of 4848 he in total. The reserve was established in 1935 in order to protect, conserve and study Imeretian oak (*Quercus imeretina* Steven ex Woronow) – a relict endemic species, and zelkova (*Zelkova carpinifolia* (Pallas) C. Koch) – another relict species. Along with these species Georgian oak (*Quercus iberica* Steven), Hartwiss' oak (*Q. hartwissiana* Steven) grow in the reserve. Small number of *Carpinus caucasica* Grossheim, *Acer campestre* Linné, *Ulmus minor* Miller, *Fraxinus excelsior* Linné are admixed to the oak stands. Understorey is made up of *Carpinus orientalis* Miller, *Rhododendron luteum* Sweet, *Rubus sp., Mespilus germanica* Linné, *Ruscus ponticus* Woronow ex Grossheim, *Crataegus monogyna* Jacquin., *Frangula alnus* Miller, etc. Names of the woody plants follow Czerepanov (1995) and Gagnidze (2005). Sword is constituted by various grasses and mosses. Beside Imeretian oak Georgian oak and zelkova occur in forest west of the reserve, while forest of Imeretian oak is spread east of it. A pure stand of zelkova persisted in the place Apkhanauri on the left bank of the river Khanistskali on the area of 1.5 he [Mamisashvili 1967; 1975; Matsaberidze 1974].

Mainly podzol soils of different texture, particularly, loamy – moderate and heavy loamy soils are spread in the reserve [Kostava 1947]. Besides, alluvial soils occur on small areas. Substrate of riverbanks in the southwestern part of the reserve are sandy with cobble-stone [Kostava 1947; Matsaberidze 1974].

Our research aimed at revealing flora of soil algae occurring on the territory of the reserve and determining their distribution peculiarities within the reserve. We studied some formations of

Georgian and Imeretian oaks in three massifs of the reserve (Adjameti, Vartsikhe, Sviri) as well as forests made up of zelkova and oaks in Adjameti and Vartsikhe.



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Materials and Methods

Samples were taken from soil surface (0-3 cm depth) directly at the bases of tree trunks. The samples taken from the nine places were treated the same year (1986) at algological laboratory of the Department of Lower Plants of Komarov Botanic Institute (St. Petersburg, Russia).

The samples were sown on Danilov's nutrient medium. Sampling and subsequent treatment techniques used are given in the book "Soil algae" [Hollerbakh & Shtina, 1969].

The algae were identified using Keys of freshwater algae of the USSR [Hollerbakh et al. 1953; Zabelina et al. 1951; Dedusenko-Shchegoleva & Hollerbakh 1962; Dedusenko-Shchegoleva et al. 1959; Moshkova & Hollerbakh 1986], also a work on the genus *Chlorella* [Andreeva, 1975], a short key of *Chlorococcales* of the Ukrainian SSR [Tsarenko, 1990], Flora of cryptogamic plants of the USSR [Kosinskaja, 1960]. Names and classification of the blue green algae are given according to Komárek & Anagnostidis (1998) and Kondratieva (1968). The others (*Chlorophyta, Xanthophyta, Bacillariophyta*) are presented according to the system of the abovementioned keys with some nomenclatural changes given in literature [Kostikov at el., 2001; Vasser (ed.), 2000].

Results and Discussion

We have revealed 81 taxa of algae from 5 divisions (see the checklist below). 37 taxa (46%) belong to the division *Chlorophyta,* 24 (30%) to *Cyanophyta,* 11 (13%) to *Xanthophyta*, 8 (10%) to *Bacillariophyta* and 1 (1%) to *Eustigmatophyta.* They are arranged in 9 classes, 20 orders, 37 families and 51 genera (Table 1).

Table 1. Taxonomic diversity of soil algae of Adjameti State Reserve

Division	Class	Order	Family	Genus	Species and intraspecific taxa	Percentage %
Chlorophyta	4	11	17	23	37	46
Cyanophyta	2	3	9	14	24	30
Xanthophyta	1	3	6	8	11	13
Bacillariophyta	1	2	4	5	8	10
Eustigmatophyta	1	1	1	1	1	1
Total	9	20	37	51	81	100

Green algae predominate in the soils of the reserve in the aspect of species number as well as abundance. Blue-green algae according to the number of the present species is the following group; however, they mainly occur in low abundance and have limited distribution. Yellow-green algae are much less diverse than blue-green algae; only some of them are present in abundance, although mostly sporadically. If the division *Eustigmatophyta* represented by a single species is not taken into account, diatoms make up the least diverse and most weakly developed group here.

Algosynusiae are relatively better formed in oak forests (Imeretian oak, Georgian oak). 79 species were recorded in these formations: 36 from *Chlorophyta*, 23 from *Cyanophyta*, 11 from *Xanthophyta*, 8 from *Bacillariophyta*, 1 from *Eustigmatophyta.* Among oak forests algosynusiae are more diverse in the Imeretian oak formations, where 73 algae were recorded. In Georgian oak formations 56 soil algae were identified (Table 2).

Among the Imeretian oak formations studied by us, the formation of Imeretian oak in Sviri was distinguished by comparatively low number of soil algae, while in Georgian oak formations the same result was mentioned in Ajameti oak formation (Table 3). This must probably be due to the fact that these formations are developed on heavy loamy soil with bad aeration and low water permeability; the understorey is made up of *Rhododendron luteum* and *Crataegus monogyna,* which form impassable thickets.

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Table 2. Distribution of algae in soils of the studied forest formations of Adjameti State Reserve

Forest formations	*Chlorophyta*	*Cyanophyta*	*Xanthophyta*	*Bacillariophyta*	*Eustigmatophyta*	Total number
Oak forest	36	23	11	8	1	79
Imeretian oak formations	35	20	9	8	1	73
Georgian oak formations	24	16	9	6	1	56
Zelkova-oak formations	24	20	8	6	1	59

59 algae were recorded in the zelkova-oak formations: 24 from *Chlorophyta*, 20 from *Cyanophyta*, 8 from *Xanthophyta*, 6 from *Bacillariophyta*, 1 from *Eustigmatophyta* (Table 2). In contrast to algosinusiae of studied oak formations, those of the zelkova-oak formations do not include representatives of the families *Leptosiraceae, Neospongiococcaceae (Chlorophyta), Schizotrichaceae (Cyanophyta), Tribonemataceae (Xanthophyta).* From the studied zelkova-oak formations the number of species is somewhat lower in Adjameti zelkova-oak formations occurring on heavy loamy soils compared to the others (Table 3). In contrast to the other zelkova-oak formations the following families were not recorded in the mentioned locality: *Scenedesmaceae, Protosiphonaceae (Chlorophyta), Pseudanabaenaceae (Cyanophyta).* In our opinion, less specific diversity of the last mentioned formation should be explained by the influence of the same factors as those acting in the Sviri formation of Imeretian oak.

Table 3. Number of algae in soils of different forest formations of Adjameti State Reserve

Taxon	Adjameti massif					Vartsikhe massif			Sviri massif
	Imeretian oak formation, sandy soil with cobble -stone	Zelkova-oak formation, sandy soil with cobble -stone	Imeretian oak formation, podzol moderate loamy soil	Georgian oak formation, podzol heavy loamy soil	Zelkova-oak formation, podzol heavy loamy soil	Imeretian oak formation, podzol moderate loamy soil	Georgian oak formation, podzol moderate loamy soil	Zelkova-oak formation, podzol moderate loamy soil	Imeretian oak formation, podzol heavy loamy soi
Chlorophyta	18	15	18	15	14	24	19	14	15
Cyanophyta	14	10	9	9	10	11	12	13	11
Xanthophyta	6	5	6	7	4	8	6	7	6
Bacillariophyta	5	5	6	4	4	5	6	4	5
Eustigmatophyta	1	1	1	1	1	1	1	1	1
Total amount	44	36	40	36	33	49	44	39	38

In the soils of the reserve a complex constituted by 17 algae was distinguished. These algae were recorded in all forest formations (see the checklist), although their abundance varied. The majority of the algae constituting the complex are representatives of green algae (8 species), in particular, species of the genera *Chlamydomonas, Bracteacoccus, Choricystis, Chlorococcum, Muriella* and *Parietochloris*. Along with the listed genera of green algae, 4 species of yellow-green

algae are in the leading complex; these are species of the genera *Botrydiopsis, Heterococcus, Xanthonema* and *Polyedriella*. From the divisions *Bacillariophyta* and *Cyanophyta* weakly developed representatives of the following genera are included in this complex: *Hantzschia, Luticola, Pinnularia* (*Bacillariophyta*), *Nostoc* and *Tolypothrix* (*Cyanophyta*). *Eustigmatos magnus,* the only recorded species of the division *Eustigmatophyta*, is in the complex of leading species.

The checklist of 81 species recorded in Adjameti State Reserve is presented in the given work. 19 of these species have not been mentioned for Georgia's soils so far; they are marked with asterisk in the checklist. 10 of them are new to Georgia's algoflora; they are marked with two asterisks. Divisions are arranged according to the scheme accepted by Kostikov et al. (2001), while genera within the divisions and species within the genera are arranged in alphabetical order. Numbers indicated in the checklist correspond to the locality, where a species was recorded.

Below a list of the studied forest formations and the checklist of algae found in Adjameti State Reserve is given. Numbers in the first line of the checklist indicate a forest formation, in which an algae was recorded: 1. Adjameti massif, the Imeretian oak formation with the understorey made up of *Carpinus orientalis, Mespilus germanica, Crataegus monogyna*, under Imeretian oak crone, sandy soil with cobble-stone; 2. Adjameti massif, the zelkova-oak formation with the understorey made up of the same woody plant species as in the place 1, under zelkova crone, sandy soil with cobble-stone; 3. Adjameti massif, the Imeretian oak formation with the understorey made up of *Rhododendron luteum, Crataegus monogyna, Ruscus ponticus*, under Imeretian oak crone, podzol moderate loamy soil; 4. Adjameti massif, the Georgian oak formation with the understorey made up of *Crataegus monogyna, Ruscus ponticus*, under Georgian oak crone, podzol heavy loamy soil; 5. Adjameti massif, the zelkova-oak formation (Imeretian oak, Georgian oak, small number of *Carpinus caucasica* admixed) with the understorey made up of *Crataegus monogyna,* under zelkova crone, podzol heavy loamy soil; 6. Vartsikhe massif, the Imeretian oak formation (in places *Carpinus caucasica* are admixed) with the understorey made up of *Crataegus monogyna, Ruscus ponticus*, under Imeretian oak crone, podzol moderate loamy soil; 7. Vartsikhe massif, the Georgian oak formation (small number of *Carpinus caucasica* admixed) with the understorey made up *Rubus spp.* and other thorny shrubs, under Georgian oak crone, podzol moderate loamy soil; 8. Vartsikhe massif, the zelkova-oak formation (Imeretian oak, Georgian oak, in places *Ulmus minor* admixed) with the understorey made up of *Rubus spp.* and other thorny shrubs, *Ruscus ponticus*, under zelkova crone, podzol moderate loamy soil; 9. Sviri massif, the Imeretian oak formation (in places *Carpinus caucasica* admixed) with the understorey made up of *Rhododendron luteum, Crataegus monogyna, Ruscus ponticus, Mespilus germanica, Rubus spp.*, under Imeretian oak crone, podzol heavy loamy soil. The intensity of algal development in soils is given with following marks: S – single, R – rarely, O – often, A – abundant, M – massively, – not found.

Checklist. Distribution of algae in different forest formations of Adjameti state reserve

Taxon	1	2	3	4	5	6	7	8	9
CYANOPHYTA									
Aphanocapsa fusco-lutea Hansgirg	–	–	–	–	–	–	S	S	–
A.incerta (Lemmermann) Gronberg et Komárek	–	R	–	–	R	–	R	R	–
Calothrix elenkinii Kossinskajas	–	–	R	–	–	–	S	S	S
Chroococcus dispersus (Keissler) Lemmermann	S	–	–	O	O	–	–	–	–
Chr. turgidus (Kützing) Nägeli*	–	–	–	–	–	–	–	R	–
Cylindrospermum licheniforme (Bory) Kützing	–	–	R	–	–	–	R	–	–
Gloeocapsa alpina Nägeli Brand	S	–	–	–	–	–	–	S	–
Gl. varia (A.Braun) Hollerbach*	R	O	–	–	–	–	–	–	O
Leptolyngbya boryana (Gomont) Anagnostidis et Komárek	S	–	–	–	–	S	S	S	S
L.foveolarum (Rabenhorst ex Gomont) Anagnostidis et	–	–	S	S	–	S	–	–	S

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Komárek									
Microcoleus vaginatus (Vaucher) Gomont	M	A	O	M	–	–	R	A	O
Microcystis pulverea (Wood) Forti in De Tonis	–	–	S	–	R	R	–	–	R
Nostoc linckia (Roth) Bornet ex Bornet et Flahault f. muscorum (Agardh) Elenkin*	O	O	–	O	O	S	–	M	–
N.microscopicum Carmichael ex Bornet et Flahault**	R	R	R	R	R	R	R	R	R
N. paludosum Kützing ex Bornet et Flahault	O	R	–	S	S	R	R	S	–
N. punctiforme (Kützing) Hariot	O	–	–	S	–	–	O	O	O
Phormidium autumnale (Agardh) Gomont	R	–	S	–	–	S	–	O	O
Ph. tenue (Agardh ex Gomont) Anagnostidis et Komárek	–	S	–	–	S	–	S	–	–
Ph. terebriforme (Agardh ex Gomont) Anagnostidis et Komárek	–	S	–	R	–	–	–	–	R
Schizothrix calcicola (Agardh) Gomont	–	–	–	–	–	M	–	–	–
Sch. lardacea (Cesati) Gomont	M	–	M	–	–	M	–	–	–
Symploca muscorum (Agardh) Gomont	M	–	–	–	M	M	–	–	–
Tolypothrix tenuis Kützing ex Bornet et Flahaults	R	R	R	R	R	R	R	R	R
Trichromus variabilis (Kützing ex Bornet et Flahault) Komárek et Anagnostidis	S	S	–	–	S	–	S	–	–
EUSTIGMATOPHYTA									
Eustigmatos magnus (B.Petersen) Hibberd	O	O	O	O	O	O	O	O	O
XANTHOPHYTA									
Botrydiopsis eriensis Snow	O	O	O	O	O	O	O	O	O
Characiopsis minima Pascher	–	–	–	–	–	S	–	S	S
Ellipsoidion anulatum Pascher	O	O	O	–	–	R	O	–	–
Heterococcus caespitosus Vischer**	–	–	–	O	–	–	–	–	–
H.moniliformis Vischer**	–	–	–	–	–	–	R	R	–
H. viridis Chodat	O	O	O	O	O	O	O	O	O
Monallantus brevicylindrus Pascher	A	–	S	A	–	–	–	–	–
Polyedriella aculeata Pascher	R	R	R	R	R	R	R	R	R
Tribonema minus (Klebs) Hazen	–	–	–	S	–	S	–	–	–
Xanthonema bristolianum (Pascher) Silva**	–	–	–	–	–	O	–	O	O
X. exile (Klebs) Silva	R	R	R	R	R	R	R	R	R
BACILLARIOPHYTA									
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve et Grunow	R	R	R	R	R	R	R	R	R
H. amphioxys f. capitata O.F. Müller	–	R	R	S	–	–	S	S	–
Luticola mutica (Kützing) Mann in Round et al.	R	R	R	R	R	R	R	R	R
Navicula minima Grunow in Van Heurck*	–	S	–	–	–	S	–	–	–
Nitzschia palea (Kützing) W.Smith	–	–	–	–	–	–	O	–	O
Pinnularia borealis Ehrenberg var borialis	R	R	R	R	R	R	R	R	R
P.borealis var.brevicostata Hustedt*	S	–	S	–	–	S	–	–	–
P. intermedia (Lagerstedt) Cleve	S	–	S	–	R	–	S	–	S
CHLOROPHYTA									
Bracteacoccus minor (Chodat) Petrová	O	O	O	O	O	O	O	O	O
Chlamydomonas elliptica Korschikov in Pascher	–	–	–	–	–	O	–	–	–
Ch. gloeogama Korschikov in Pascher	O	O	O	O	O	O	O	O	O
Ch. oblonga Pringsheim	–	–	–	–	–	R	–	–	R
Ch. oblongella Lund	A	A	A	A	A	A	A	A	A
Ch. proboscigera Korschikov in Pascher var. proboscigera**	R	–	–	–	–	–	–	–	–
Ch. proboscigera var.conferta (Korschikov) Ettl	S	–	–	S	–	–	–	–	–
Ch.reinhardtii Dangeard*	–	–	–	–	–	S	R	–	S
Ch. stellata Dill	–	S	S	S	–	–	–	–	–
Chlorella mirabilis Andreeva**	S	–	–	–	S	–	–	–	–
Chlorococcum echinozygotum Starr**	–	–	–	–	–	S	S	–	–
Ch.sp.	A	A	A	A	A	A	A	A	A
Chlorosarcinopsis minor Herndon	S	S	O	–	–	–	–	S	–
Choricystis minor (Skuja) fott	O	O	O	O	O	O	O	O	O

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Closterium sp.*	–	S	S	–	–	S	S	–	–
Coelastrum sp.	–	S	S	–	–	S	S	–	–
Dictyococcus varians Gerneck emend. Starr	–	–	S	–	O	O	–	O	–
Gloeotilia protogenita Kützing	–	–	–	–	–	–	–	S	–
Klebsormidium dissectum (Gay) Ettl et Cärtner	–	–	–	–	O	–	O	–	O
K. flaccidum (Kützing) Silva et al.	R	–	O	–	–	–	–	–	–
K. nitens (Meneghini in Kützing) Lokhorst	–	–	–	–	O	S	–	–	–
Leptosira terricola (Bristol) Printz	–	–	–	O	–	O	S	–	–
Microspora quadrata Hazen	O	–	–	–	–	–	–	O	–
M. tumidula Hazen	R	–	–	–	–	R	R	–	R
Muriella magna Fritsch et John	R	R	R	R	R	R	R	R	R
M.terrestris B.Petersen**	–	–	–	S	S	S	–	–	–
Neospongiococcum sp.**	–	–	–	–	–	O	O	–	A
Parietochloris alveolaris (Bold) Watanabe et Floyd	R	R	R	R	R	R	R	R	R
Pseudococcomyxa sp.**	M	–	M	–	–	–	O	–	M
Scenedesmus quadricauda (Turpin) Brébisson. sensu Chodat*	–	R	R	R	–	–	–	–	–
Spongiochloris incrassata Chantanachat et Bold	O	O	–	–	–	–	–	–	–
S. minor Chantanachat et Bold	–	–	O	–	–	R	S	–	–
Spongiococcum tetrasporum Deason	–	–	–	O	–	–	O	–	–
Stichococcus bacillaris Nägeli	–	–	–	O	–	O	–	S	O
Tetracystis elliptica Nakano	O	O	O	O	–	R	O	A	–
Ulothrix tenerrima Kützing*	–	S	–	–	S	S	–	–	–
U. variabilis Kützing	S	–	O	–	R	O	–	R	S

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მონაცემები აჯამეთის სახელმწიფო ნაკრძალის ნიადაგის ალგოფლორის შესახებ

კუხალეიშვილი ლ.

ნიკო კეცხოველის ბოტანიკის ინსტიტუტი

(მიღებულია 16.07.2007)

რეზიუმე

შესწავლილია აჯამეთის სახელმწიფო ნაკრძალის იმერული და ქართული მუხის ზოგიერთი ფორმაცია, აგრეთვე ძელქვნარ-მუხნარები. გამოვლენილია 81 წყალმცენარე ხუთი განყოფილებიდან: Chlorophyta - 37(46%), Cyanophyta - 24(30%), Bacillariophyta - 8(10%), Eustigmatophyta - 1(1%). ისინი განაწილებულნი არიან 9 კლასში, 20 რიგსა, 37 ოჯახსა და 51 გვარში. შესწავლილი ნიადაგებიდან ალგოსინუზიები უკეთაა წარმოდგენილი მუხნარებში (იმერული, ქართული), სადაც 79 წყალმცენარე დაფიქსირდა, ძელქვნარ-მუხნარებში კი 59 წყალმცენარე აღმოჩნდა. ნაკრძალის წყალმცენარეებიდან 19 ადრე საქართველოს ნიადაგებისათვის არ ყოფილა მითითებული, ხოლო მათ შორის 10 ახალია საქართველოს ალგოფლორისათვის.

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STATISTICAL ANALYSIS OF FLORA OF KIZIKI (EAST GEORGIA)

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Abstract

The floristic composition of Kiziki (East Georgia) was studied. 1103 species of 550 genera and 115 families of vascular plants are recorded. The most numerous are angiosperms represented by 1084(98.3%) species; among them 877(79.5%) are **dicotyiedoneae** and 207(18.8%) – **monocotyledoneae. Pteridophytes** and **gymnosperms** are represented by a small number of species - 12(1.1%) and 7(0.6%) species, accordingly. The leading families in floristic spectrum are: ***Poaceae*** - 115(10.4%), ***Asteraceae*** - 113(10.2%), ***Fabaceae*** - 104(9.4%), ***Brassicaeae*** - 64(5.8%), ***Lamiaceae*** - 47(4.3%), ***Chenopodiceae*** - 43(3.9%), ***Caryophyllaceae*** - 41(3,7%), ***Apiaceae*** - 41(3.7%), ***Rosaceae*** - 39(3.6%), ***Boraginaceae*** - 36(3.3%) species. 1-10 families embrace 643 species (58.3%) of the whole floristic composition. Floristic spectrum of Kiziki is original – it does not fit the standard frames of any floristic centre. However, the influence of various floristic centres (Mediterranean, front Asia, Turan, etc.) is shown. Floragenesic relationship with Irano-Turanian floristic centres is designated in the southern part of Kiziki, but in the North direction the influence of Mediterranean is getting stronger. Thus, the low level of endemism in Kiziki (5.3%) is quite natural.

Key words: flora, family, genera, species, Irano-Turanian, Mediterranean area.

Introduction

Kiziki represents the extreme east part of Georgia and consists of two administrative districts – Sighnaghi and Dedoplistskaro. Physical-geographically it is the part of Iveri region. Geographic areas having various physical-geographic structure and geological origin are represented on its territory. Unofficially, the extreme south-eastern part (Eldari lowland) is the part of Mtkvari-Araxi lowland; the great part of the territory belongs to Iori plateau. South-eastern ends of Gombori range (part of Chotori-Ozaani) and east part of Alazani plain also resists on the territory of Kiziki. The altitude of Kiziki ranges from 90(100)m (south-eastern part of Eldari lowland) to 1084m (mountain Chotori). The territory of Kiziki is characterized by complex topography. Various anticline ranges and low ranges alternate with depressive plains located between these two ranges [Maruashvili, 1964; Khachidze, 1985].

Zonal change of climate from the half-dry subtropical desert to the modularity damp climate is noticed towards South-north. The average annual precipitation to the same direction grows from 250 mm to 600 mm and more, evaporation reduces from 900-1000 mm to 700-800

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mm, accordingly moistening coefficient grows from 0.3-0.4 to 0.6-1.0. The average annual temperature reduces from 14.2 °C – to 10.5 °C [Atlas of Georgian SSR, 1964, Khachidze, 1985].

Different soil types are formed in Kiziki and their distribution mainly bears zonal character. From the South to the North spatial distribution of soils is as follows: 1. desert and semi-desert loamy and clayey grey soils with variable degree of salinity; 2. arid forest cinnamonic, chestnut and brown soils; 3. chernozem and chernozem-like soils of different thickness; 4. forest cinnamonic, humus-carbonate and cinnamonic soils. Meadow cinnamonic, solonchak, solonetz, meadow black and saline meadow black soils are intrazonally spread on the Alazani plain. In riparian forests of the rivers Alazani and Iori alluvial soils are formed. South-facing steep slopes of monoclinal low ranges located in the south-eastern part of the Iori upland, massifs of Mijniskure and Usakhelo-Mta are worth special notice. They are mostly devoid of soil cover and are represented by bare mother rocks, cemented conglomerates, sandstone and scree formed on airing peel and saline loam, clay and clay-sand badlands [Maruashvili, 1964; Sabashvili 1965; Soil map of Georgia, 1999].

Vegetation of Kiziki is very diverse. Main biomes are: deserts (plain and foothill deserts), arid woodland forests, phryganoid vegetation, steppes, foothill (and partly lower mountain belt) deciduous forests, summer-green hemixerophilous shrubbery of the shibliak type, riparian forests and saline plains. Besides, we meet phytocenoses of *Phragmites australis*, *Arundo donax* and meadow-steppes, also various xerophilous complexes [Khachidze, 1985; Lachashvili, 1989; Nakhutsrishvili, 1999; Lachashvili, Khachidze, 2000 ect.].

According to R.Gagnidze (2004) Kiziki is considered within botanic–geographic province of the East of South Caucasus (Iveria) of sub-Mediterranean region of ancient royal Mediterranean area.

The aim of our research was to determine the floristic composition of Kiziki according to own and literature data; the systematic analyses; and to ascertain its florogenetic relationships.

Materials and Methods

The floristic material was collected during 1984-1991 and 2004-2006 by route and stationary methods. Geographic elements correspond to the methods and principles of R.Gagnidze (2004). Latin names of taxa correspond to S.K. Czerepanov (1995).

Results and Discussion

It is very important to determine the number of principle taxa (families, genera, species) of studied region and calculate their ratio during studies of regional floras [Tolmachev 1986]. Such statistical analysis enables to estimate the structure and diversity of region flora.

About 1103 species of vascular plants (pteridophytes, gymnosperms and angiosperms or flowering plants) belonging to 550 genera and 115 families are distributed in Kiziki. The most numerous are angiosperms; they are represented by 1084 species (98.3%*), among them 877 (79.5%) are dicotyledonous and 207 (18.8%) – monocotyledonous (Table 1). Pteridophytes and gymnosperms are represented by a small number of species, in particular, by 12 (1.1%) and 7 (0.6%) species. However, coenotic importance of gymnosperms in the composition of the main type vegetation of Kiziki - arid forests, is great.

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Table 1. Whole floristic spectrum of Kiziki

Taxa of higher rank	Family		Genera		Species	
	Quantity	%*	Quantity	%**	Quantity	%***
Pteridophita	6	5.22	7	1.3	12	1.1
Gimnospermae	2	1.74	3	0.5	7	0.6
Angiospermae among them:	107	93.04	540	98.2	1084	98.3
Dicotyledoneae	88	76.52	432	78.6	877	79.5
Monocotyledonaea	19	16.52	108	19.6	207	18.8
Total	115	100	550	100	1103	100

- calculated according to the whole number of the vascular plant: families - * %; genera - ** %; species - *** %.

The numbers of genera in Kiziki flora are disproportionately distributed among the families. 92 families from 115 families are represented only by – 1-3 genera (51 families-by 1, 33 families-by 2, and 8 families-by 3 genera each). The leading 1-10 families (Table 2) comprise 60.5% (333 genera) of the whole number of genera, and families 1-19 comprising more than an average number of genera (5 genera) embrace 71.5% (393 genera) of the whole number of genera. The rest 38,5% (157 genera) are distributed within 96 families. It should be mentioned that great number of families are represented by only 1-3 genera (51 families-by 1, 33 families-by 2 and 8 families-by 3 genera each).

Table 2. Number of genera of the 1-10 leading families

Family	Number of genera	%*
1. *Poaceae*	65	11,8
2. *Asteraceae*	64	11,6
3. *Brassicaceae*	39	7,1
4. *Apiaceae*	33	6
5. *Fabacea*	30	5,5
6. *Lamiaceae*	22	4
7.*Boraginaceae*	21	3,8
8. *Rosaceae*	21	3,8
9. *Chenopodiaceae*	20	3,6
10. *Caryophyllaceae*	18	3,3
Total	333	60,5

* % - calculated according to the whole number of the vascular plant genera

The same families are leading according to the number of species as the families occupying leading positions according to the number of genera; however, sequence is somewhat different (Table 3). 1-10 families embrace 58.3% (643 species) of the vascular plants. Families, each comprising more than a half of the average number of species (10 species) per family (1-19 families) cover 73% (805 species) of the whole number of species. The other 96 families comprise only 298 species (27%). It is worth mentioning, that more than the half families (67 families) are represented only by 1-3 species (25 families-by 1, 25 families-by 2 and 17 families-by 3 species each).

Such portion of 1-10 families is characteristic to the floras of typical Mediterranean Area [Tolmachev, 1986; Gagnidze, Davitadze, 2000], however, the correlation of each region of Kiziki is different. South regions - Eldari-Chatma plains (Families 1-10 embrace 66.1% of vascular plants) and south-eastern part of Iori plateau (62.1%) are most contrasting [Lachashvili, 1989; Lachashvili I. et al., 2004], but in some ecosystems (e.g. in deserts) this index is about 80%. Such increased contrasting is characteristic to Irano-Turan floras [Bikov, 1978; Tolmachev, 1986; Kamelin,

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Bochantsev, Gorelova, 1989]. In the North direction the contrasting decreases. Such conformity is connected with the changes of physical-geographic conditions – living environment in the south part of Kiziki is quite extreme, but towards North it is getting "weak".

Table 3. Number of species by 1-10 leading families

Family	Number of species	%*
1. *Poaceae*	115	10,4
2. *Astrraceae*	113	10,2
3. *Fabaceae*	104	9,4
4. *Brassicaeae*	64	5,8
5. *Lamiaceae*	47	4,3
6. *Chenopodiaceae*	43	3,9
7. *Caryphyllaceae*	41	3,7
8. *Apiaceae*	41	3,7
9. *Rosaceae*	39	3,6
10. *Boraginaceae*	36	3,3
Total	643	58,3

* % - calculated according to the whole number of the vascular plant species.

Genera in the flora of Kiziki are "poor" in species. Only 27 genera comprise 6-17 species (Table 4), whereas the majority (330 genera) is represented by 1 species each. Thus, it may be concluded that more or less large quantity of species of Kiziki flora is provided by relatively large number of genera, because the most part of them is represented by 1-2(3) species.

Table 4. Number of species by genera

Genera	Number of genera
Trifolium	17
Veronica	14
Vicia	14
Carex	13
Astrogalus	12
Euphorbia	10
Geranium	10
Medicago	9
Galium	8
Centaurea	8
Lathyrus	8
Ranunculus	8
Silene	7
Atriplex	7
Chenopodium	7
Viola	7
Gagea	7
Poa	7

Genera	Number of genera
Salvia	7
Stipa	7
Cerastium	6
Plantago	6
Poligonum	6
Rumex	6
Verbascum	6
Campanula	6
Linum	6
In the rest:	
22 genera	5-5
21 genera	4-4
50 genera	3-3
100 genera	2-2
330 genera	each

1-10(15) families, which are leading according to the number of species constituting them, are considered very important in floristic studies conducted in any region. In this aspect floristic spectrum of Kiziki is especially interesting, non-standard and original. It does not fit a standard pattern of any floristic centre; however, the influence of various floristic centres (Mediterranean area, Front Asia, Turan and est.) is shown. Particularly, high position of families: ***Fabaceae, Brassicaceae, Caryophyllaceae*** refers to the influence of the Mediterranean floristic centre [Tolmachev, 1986; Gagnidze, 2000]; further, it is worth attention that a family ***Asteraceae*** always occurring on the first place in Mediterranean floras [Tolmachev, 1986], occupies the second

position in the spectrum of Kiziki. Presence of the family ***Lamiaceae*** among the first 10 families points to the relation with Front Asia and Middle Asia centers [Faivush, 1986]. High position of families ***Chenopodiaceae and Boraginaceae,*** which is a characteristic to Irano-Thuranian desert, is also worth mentioning [Rodin, 1961; Bikov, 1978; Kamelin, Bochancev, Gorelova, 1989]. On this background 9th position of the family ***Rosaceae,*** which is widespread in the Holarctic kingdom appears unordinary. This family generally does not fall into the first 10 families in the spectra of the typical Mediterranean floras [Turril, 1929; Tolmachev, 1986; Faivush, 1989]; but in Irano-Thuranian floristic spectra it passes on lower positions in floras of non-forested regions compared to those of forested ones [Tolmachev, 1986; Faivush, 1986].

For the floristic structural analysis of Kiziki, it is worth attention the families being on the 11-15 positions, particularly, 11th place of the family ***Scrophulariaceae*** (32 species). It is widespread almost all over the world [Mabberley, 1998; Kadereit, 2004]. Especially its majority is represented in the mountainous regions of the moderate warm and subtropical regions. Besides, it represents one of the characteristic families of Mediterranean flora [Tolmachev, 1986]. In Georgia it is the leading family in the flora of mountainous and mainly more or less humid regions (Svaneti, Racha-Lechkhumi, Kartly - pools of Aragvi-ksani, Khevi, and Shida Kakheti) [Lachashvili, Mamukelashvili, 1986; Gagnidze, Davitadze, 2000]. In floristic spectrum of Kiziki its comparatively high position is mainly underlined by the deciduous forests and secondary meadow-steppe ecosystems of the foothills (and lower mountain belt) in the North-western and central parts of the region, and by the influence of the neighbouring humid Kakheti Caucasus. It is worth attention that in Kiziki flora ***Veronica*** is the leading genus according to the number of species (14 species, 2-3 places). 12th place of the family ***Cyperaceae*** (28 species) is characteristic to humid and hyper humid ecosystems. Its high position in the floristic spectrum of Kiziki is related on the one hand with the moist plains along the riverside and on the other hand with the foothills and deciduous forests of the downhill belt. Among the leading genera the genus ***Carex*** with 13 species occupies 4th place. These two families consisting 9 by 9 genera occur on the leading position (11-12 places). Second 10 families are: ***Rubiaceae*** (12 species), ***Ranunculaceae*** (20), ***Polygonaceae*** (14), ***Geraniaceae*** (13), ***Malvaceae*** (12), ***Euphorbiaceae, Orchidaceae*** (11-11). Majority of ***Polygonaceae***'s representatives spread in Kiziki are characteristic plants to the humid and hyper humid places and we mainly meet them along the rivers and irrigation canals.

Family ***Liliaceae*** should be mentioned separately. It is represented by 9 species in the flora of Kiziki and is distributed only on 20-23 places. If we discuss it by old understandings (including families ***Asparagaceae*** – 2 species, ***Aspodelinaceae*** – 2, ***Colchicaeae*** – 2, ***Convallariceae*** – 4, ***Hyacinsthaceae*** – 8, ***Smilacaceae*** – 1) it will appear on the 12-13 positions by 28 species, which should be normal for the flora of Kiziki.

However, distribution of families according to the separate regions of Kiziki is not the same. From the south to north in parallel with zonal changes of physical-geographic conditions we notice some important changes in floristic spectrum; e. g. ***Chenopodiaceae*** takes the leading place in the south part of Kiziki – in the flora of south-east part of Iori-plateau (e. g. Vashlovani State Reserve) and Eldari lowland [Lachashvili, 1989; J. Lachashvili, N. Lachashvili, M. Khachidze, 2004]. But in the central and north-western parts species of this family are not represented, or we meet only 1-2 species. Besides, family ***Boraginaceae*** is on the higher position in described regions (9 and 6 places) than in the common spectrum of flora in Kiziki. Accordingly, influence of Irano-Turanian deserts is mainly spread on the flora of the south and partly of the east part of Kiziki. Decreasing portion of ***Liliaceae*** from the south direction to the north is also worth attention, which should be normal. At the same time portion of families ***Scrophulariceae*** and ***Rosaceae*** increases to the north; it is also worth mentioning that great part of the representatives of these families (in case of ***Scrophulariceae*** 62% and ***Rosaceae*** 51%) are spread only in the central, north-west and north

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parts of the region. All these facts indicate the influence of the woody ecosystems of Caucasus range of Kakheti and partly of the floristic centre of Mediterranean area.

Areas of Dedoplistskaro take floristically distinguished and special place in Kiziki, where the limestone massifs of Jurassic period are represented. Structure of flora and vegetation communities of these limestone massifs is obviously of Mediterranean area, which was noted earlier by M. Sakhokia (1958). It is important that local endemics of Kiziki (***Campanula kachetica*** and ***Galium praemontanum)*** are spread right here.

Geographic elements represented in the flora of Kiziki indicate the influence of various floristic centres. In the structures of the studied flora the following geographic elements are important: Mediterranean* - 248, Palearctic – 200, Mediterranean-Irano-Thuranin – 115, Caucasian-Front Asian – 110, Caucasian-Minor Asian – 85, Holarctic – 78, Caucasian-Minor Asian-Front Asian – 75, European-Mediterranean – 70, Minor Asian-Front Asian – 69, European – 34, Pancontinental (cosmopolite) – 24 and other geographic elements. Geographic elements of Kolkheti-Hirkani (3 species) and Hirkani (1) are also represented. Such diversity of geographic elements talks about the richness and habitude of Kiziki flora.

We suppose that the represented spectrum of species and geographic elements are in accordance with the law of nature and is determined by geographic location of Kiziki together with other environmental conditions. The studied area belongs to the central part of the south Caucasus located at the intersection of different floristic centres and therefore is subjected to more or less strong influence of each of these centres. This influence is reflected in the structure of Kiziki flora. Historical and geological period of the region also played important role.

Thus, the low level of endemism in Kiziki flora (5.3%) is quite natural. 51 endemic species of Caucasus and 8 – of Georgia are spread on the territory, among them 2 are endemics of Kiziki. Despite of the low percentage index we suppose that 59 species of the region located at the intersection of different floristic centres and being under their influence should not be considered as the low index.

Kiziki endemics:

Campanula kakhetica,
Gallium praemontanum

Georgian endemics:

Onobrychis kakhetica
Paeonia carthalinica
Paeonia mlokosewitschii
Psephelus carthalinicus
Pyrus sakhokiana
Ulmus georgica

Caucasian endemics:

Acantholimon fominii
Alcea sosnovskyi
Anthyllis lachnophora
Asparagus caspius
Astragalus sphaerocephalus
Bellevalia montana
Berberis iberica
Bupleurum wittmann
Crataegus caucasica
Dianthus inamoenus
Dianthus subulosus
Erysimum aureum
Ficaria ledebourii
Gagea helenae
Galanthus lagodechianus
Galatella eldarica
Galium eldaricum
Gypsophila steveni
Heracleum sosnowskyi
Iris carthalinicus
Iris iberica
Isatis iberica
Jurinea blanda
Lotus caucasicus
Melampyrum caucasicum
Onobrychis cyri

* Groups of geographic distribution ranges are considered in *sensu lato*

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Onobrychis iberica
Onobrychis komarovii
Ophris caucasica
Paeonia caucasica
Pimpinella aromatica
Primula woronowii
Pyrus georgica
Ranunculus caucasicus
Reseda globulosa
Rubia transcaucasica
Salvia garedji
Scabiosa georgica
Scorzonera biebersteinii
Seseli grandivitatum
Sosnovskia ambliolepis
Symphytum caucasicum
Taraxacum praticola
Teucrium nuchense
Thalictrum buschiorum
Thymus karjaginii
Thymus tiflisiensis
Torularia eldarica
Tragopogon tuberosus
Tulipa eichleri
Vicia ciliatula

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ქიზიყის (აღმოსავლეთ საქართველო) ფლორის სტატისტიკური ანალიზი

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(მიღებულია 18.07.2007)

რეზიუმე

შესწავლილია ქიზიყის ფლორისტული შემადგენლობა. აღირიცხა ჭურჭლოვან მცენარეთა 115 ოჯახის, 550 გვარის 1103 სახეობა. ყველაზე მრავალრიცხოვნად ფარულთესლიანი მცენარეებია წარმოდგენილი – 1084 სახეობა (98.3%). მათგან ორლებნიანები – 877 (79.5%), ერთლებნიანები – 207 (18.8%). უმნიშვნელოა გვიმრანაირებისა (12 სახეობა; 1.1%) და შიშველთესლიანების (7; 0.6%) რაოდენობა. ფლორისტულ სპექტრში წამყვანი ოჯახებია: *Poaceae* – 115 სახეობა (10.4%), *Asteraceae* – 113(10.2%), *Fabaceae* – 104(9.4%), *Brassicacea* – 64(5.8%), *Lamiaceae* – 47(4.3%), *Chenopodiaceae* – 43(3.9%), *Caryophyllaceae* – 41(3.7%), *Apiaceae* – 41(3.7%), *Rosaceae* – 39(3.6%), *Boraginaceae* – 36(3.3%). 1-10 ოჯახის წილად მოდის საერთო ფლორისტული შემადგენლობის 58.3% (643 სახეობა). ქიზიყის ფლორის სპექტრი არასტანდარტული და ორიგინალურია – იგი არ თავსდება არც ერთი ფლორისტული ცენტრის სტანდარტულ ჩარჩოებში, თუმცა სახეზეა სხვადასხვა ფლორისტული ცენტრის (ხმელთაშუაზღვისპირეთი, წინა აზია, თურანი და სხვ.) ზეგავლენა. ქიზიყის სამხრეთ ნაწილში გამოკვეთილია ფლოროგენეზისური კავშირები ირან-თურანის ფლორისტულ ცენტრთან, ჩრდილოეთის მიმართულებით კი ძლიერდება ხმელთაშუაზღვისპირეთის ზეგავლენა. ზემოთქმულიდან გამომდინარე კანონზომიერია ქიზიყის ფლორის დაბალი ენდემიზმი (5.3%).

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FLUORIMETRIC QUANTIFICATION OF PROTEIN IN THE CENTRAL CELL OF ARCHEGONIUM OF *GINKGO BILOBA* L.

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Abstract

Total protein content was measured in the developing central cell of archegonium by a fluorimetric assay using specific Procion Yellow 4BS binding method. Sequential changes in protein accumulation are compared to the changes occurred during cell and nucleus growth.

Key words: Fluorimetry, Procion Yellow 4BS, protein content, *Ginkgo*, female gametophyte, central cell.

Introduction

Female gametes of Gymnosperms belong to the biggest cells in the superdivision of Spermatophyta. The egg cells of some cycads reach a length of 6 mm with a diameter of 2 mm [Chamberlain, 1925]. Another feature of these cells is the prolonged period of development and maturation. Thus, in primitive Gymnosperms like Cycadales the interval between formation of the central cell (female progamete) and fertilization is more than 5 month. [Chamberlain, 1935]. In *Ginkgo biloba* L., which is the unique extant representative of order Ginkgoales, period between the origin of central cell and fertilization takes about 2 months. Here megagametogenesis can be divided into two kinds of events: those associated with the intensive growth of newly forming central cell and those that occur later during maturation of progamete when it undergoes several structural changes which prepare egg cell to be fertilized. While the species has been the subject of numerous studies concerning its evolution [Hasebe, 1997; Zhou, 1997; Zhou, Zhen 2003; Burleigh, Mathews, 2004] and general aspects of embryology [Favre–Duchartre, 1956; Shakarishvili, 1992], relatively little is understood about the biochemical parameters that control the megagametogenesis.

The purpose of the present study is twofold: first, to examine the protein accumulation in the central cell by a fluorimetric assay using specific Procion Yellow 4BS binding. Secondly, based on that analysis, to compare biochemical changes with the cell growth kinetics.

The fluorescent dichlorotriazinyl dye Procion Yellow first found use in biology as a label for newly forming bone [Fazekas de St. Groth et al., 1963; Goland, Grand, 1968]. This dye has been shown to have exceptional value as an intracellular marker of neurons [Payton, 1970; Bradford, 1976]. The Procion dyes are anionic compounds and all contain either a monochloro- or dichlorotriazinyl ring. Under alkaline conditions they are known to react with compounds

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containing an amino or hydroxyl group to form a covalent conjugate with the elimination of hydrogen chloride. Potential of Procion Yellow to make precise neuroanatomical localization has led to its widespread use in quantitative cytochemistry of proteins [Pontoppidan, Kannangara, 1994; Richards et al., 2003].

There is no available data concerning the use of Procion Yellow 4BS for protein assay in Gymnosperms female gametes. In this paper we have examined the binding of Procion Yellow 4BS to proteins of female progamete of *Ginkgo biloba* L. at key stages of its development.

Materials and Methods

Micropilar parts of the female gametophytes with archegonia were fixed in a 3:1 (v:v) solution of 75% ethanol and glacial acetic acid for 1 h, dehydrated through ethanol series (75, 90 and 2 X 100%) and xylol. Material was embedded in paraffin and sectioned according to standard procedure [Berlin, Mikshe, 1976]. Sections were stained with freshly prepared 0.1% solution of Procion Yellow 4BS on 0.1 M phosphate buffer, pH=5.6 at 56° C for 1h. Unbound dye was removed by washing preparations twice in buffer [Ivanov, 1982]. The fluorescence was measured under a Leitz-Ortoplan cytofluorimetric microscope equipped with a xenon lamp, λex=365nm, λem=585nm, objective x 70, water immersion. All data are presented as means ± se.

Results and Discussion

Cytofluorimetric estimation of protein quantity revealed the accumulation of proteins in the central cell of archegonium during its development and maturation (Fig.1). Total increase per section was 6.5 times for cell and 13.5 times for nucleus, whereas cell and nucleus sizes increased in 5.1 and 6.8 times respectively according to the area of medial section (Fig. 2 a,b). Protein accumulation rate was nonuniform and even decreased both in cytoplasm and nucleus when central cell shifted into slow growth phase. Although protein content enhanced and became markedly higher before the division of the central cell.

Different relationships were revealed between protein accumulation in the nucleus and its growth: significant protein concentration was not observed at the beginning of the intensive nuclear growth phase but occurred at the end of this period. Comparison of nucleus and nucleolus growths has revealed diminishing of nucleolus (Fig. 2 c) coincided with the decrease of protein content in cytoplasm. However considerable protein accumulation took place at the last stages of the maturation of the central cell in the absence of nucleolus. These data can be explained as by the probable involvement of pyroninophylic nucleolus-like bodies in cytoplasm as due to the entrance of proteins from the outside the central cell. As it was shown [Maugini, Fiordi, 1970] a large cytoplasmic blocks (compartments) are transported to the central cell from the jacket layer.

Relationship between cell growth and protein accumulation revealed one more peculiarity: increasing of the cell size slightly distinguished from the increasing of protein quantity in it, while the nucleus accumulated rather more protein than increased nuclear size. Such accumulation of nuclear proteins has been investigated in a number of specialized cells of plants and animals [Kafiani, Kostomarova, 1978; Barantseva, 1982]. It can be suggested that intranuclear synthesis takes place, but most probably, it could be the result of the increase of protein transport activity from cytoplasm. In the last case the nucleus plays a role of intracellular acceptor, but the means of such acceptation so far is unclear. We suppose, that accumulation of protein in nucleus of central cell has probable reason for the formation of the cytoplasm at the early stages of divisions of zygote.

The measuring of protein concentration revealed its unequal distribution at all time points. We observed the higher concentration in the chalazal region both in nucleus and cytoplasm. Relation between chalazal and micropilar concentrations was 1.8 at the early stages, but shortly before the division of the progamete it was diminished up to 1.4. Such biochemical polarization is reflected on the postzygotal events: chalazal pole becomes the site of the proembryo development.

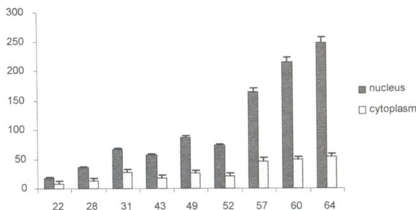

Fig. 1. Protein accumulation in the central cell of archegonium: OX- days of the observation. OY – protein content (arbitrary unit).

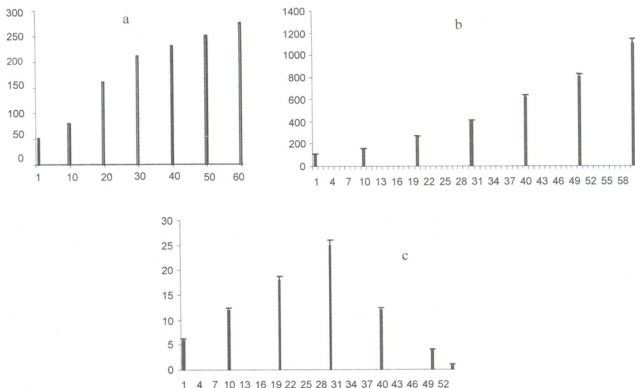

Fig. 2. Growth kinetics of the central cell: a - cell, b – nucleus, c – nucleolus. OX –days of the observation, OY –area of the median section (arbitrary unit).

In conclusion it must be noted, that the protein accumulation is not coincide in time with growth kinetics of the central cell due to special character of terminal differentiation of progamete. At the biochemical level maturation *inter alia* is expressed in the accumulation of proteins both in

the cell nucleus and cytoplasm. The division of the central cell to form the egg and ventral canal cell occurs shortly before fertilization, so that the development of the egg is almost entirely the development of the central cell. It must be emphasized that postzygotal development is rather rapid: proembryo formation takes about one week. Based on these data we suggest that the prolong period of progamete development (more than 60 days) is necessary as for "housekeeping" needs of progamete as for successful proceeding of the postzygotal events.

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ცილების რაოდენობრივი განსაზღვრა ფლუორიმეტრული მეთოდით *Ginkgo biloba*-ს არქეგონიუმის ცენტრალურ უჯრედში

შაქარიშვილი ნ.[1], ერმაკოვი ი.პ.[2]

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[2]მ. ლომონოსოვის მოსკოვის სახელმწიფო უნივერსიტეტი, მცენარეთა ფიზიოლოგიის კათედრა

(მიღებულია 2.07.2007)

რეზიუმე

შესწავლილია საერთო ცილების აკუმულაციის დინამიკა გინკგოს მდედრობით პროგამეტაში – ცენტრალურ უჯრედში მისი მომწიფების პერიოდში. გამოყენებულია სპეციფიკური ფლუორესცენტური საღებავი ყვითელი პროციონი 4BS. მიღებული მონაცემები განხილულია პროგამეტის უჯრედის, ბირთვისა და ბირთვაკის ზრდის კინეტიკასთან მიმართებაში.

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Immunology

HBV SPREADING AMONG SEAFARERS AND HUMORAL IMMUNITY INDEXES OF HBsAg CARRIERS

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Abstract

The spreading of HBV among Georgian seafarers and their humoral immunity via quantitative determination of immunoglobulins was researched. We have examined peripheral blood of 1165 seafarers and about 6.5% of them (76 male) were inactive HBsAg carriers. An absolute evidence of humoral immunity decrease among inactive carrier seafarers was shown. The reason of high percentage of inactive carriers among seafarers is likely immunodepression caused by special conditions of labor on board.

Key words: immunosorbent assay (ELISA), immunoturbidometry method, immunoglobulins IgA and IgG

Introduction

Hepatitis B is an important healthcare problem worldwide [Davis, 2002; Maddrey, 2000; Palmer, 2004]. Approximately 30 % of the world population are carriers of HBV serological markers. More than 350 million people are chronically infected by HBV and approximately one million die from the late complications each year worldwide [Chisari, Ferrari, 1995; Ferrari et al., 2003; Ganem, Prince, 2004].

The clinical spectrum of HBV infection is acute, chronic with virus replication (HBeAg positive), chronic without virus replication (HBeAg negative) and inactive HBsAg carrier state [Ganem, Prince, 2004].

Hepatitis B virus infection can be influenced by many factors, including age at infection, viral factors (HBV genotype, viral mutations, level of HBV replication), host factors (gender, age, and immune status), and exogenous factors such as concurrent infection with other hepatotropic viruses or alcohol [Lok et al., 2001; Lok et al., 2001a].

Majority of patients do recover from the acute infection, however, those who progress to chronic disease state are at the great risk of developing complications such as hepatocellular carcinoma, cirrhosis and liver failure. With the development of chronic infection 70-90% of HBsAg carriers go to the inactive carrier state (previously known as the healthy carrier state) [Pawlotsky, 2004]. The inactive carriers have no symptoms, normal liver chemistry test results, nonexistent or low levels of HBV DNA in PCR-based assays and normal or minimally abnormal liver biopsy results [Ganem, Prince, 2004]. Inactive carriers considered themselves practically healthy, in spite of this they remain infectious to others through parenteral or sexual transmission and according to some reports by towels, tooth brush, especially in circumstances such as a board

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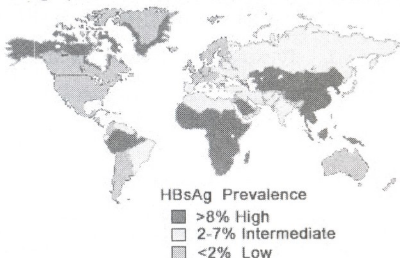
of ship. Approximately 45% of the global population live in areas with high prevalence of chronic HBV infection (>8% of the population is HBsAg-positive). In high prevalence areas, the lifetime risk of HBV infection is >60% [Davis, 1999; Kane, 1996], Georgia refers to such region (see map below).



According to some reports HBsAg spreading among seafarers is higher than in the general population. The aim of our investigation was to research the spreading of HBV among Georgian seafarers and to study their humoral immunity via quantitative determination of immunoglobulins.

Materials and Methods

We have examined peripheral blood of 1165 seafarers, all of them were male, aged 25-45.

Detection of HBV serological markers.

The blood samples were analyzed for HBsAg (surface antigen of HBV). Samples which tested positive for HBsAg were also tested for revealing of acute disease markers (anti-HBcIgM, anti-HBcIgG and HBeAg). These procedures were performed by enzyme-linked immunosorbent assay (ELISA).

The quantitative determination of human immunoglobulins IgA and IgG.

We tested HBsAg positive seafarers (inactive carriers) for humoral immunity using immunoturbidometry method by quantitative determination of IgA and IgG.

Results and Discussion

We tested the total number of 1165 seafarers. About 6.5% of them (76 male) were inactive HBsAg carriers (Fig. 1). Inactive carrier state was diagnosed as HBsAg positively, HBeAg negativity, undetectable or low HBV DNA level, normal aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels and no symptoms.

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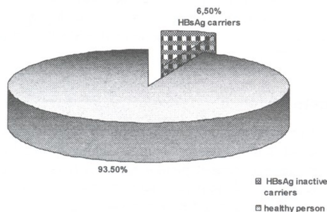

Fig. 1. Distribution of HBsAg among seafarers

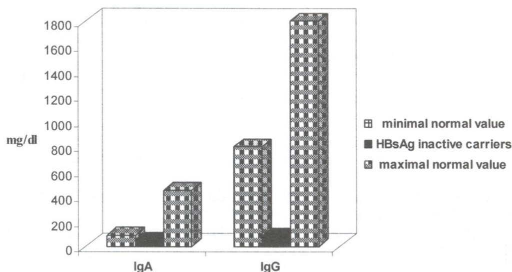

Fig. 2. Concentration of immunoglobulins IgG and IgA in blood of HBsAg carrier seafarers

The outcome of HBV infection and the pathogenesis of the attendant liver diseases are determined by immune-mediated interactions between infected host and virus. Antiviral immune response has two forms: cellular immunity and humoral immunity. Cellular immunity is worked out by T cells and humoral immunity by antibodies. The complete analysis of immunity includes T cell (cellular immunity) and B cell (humoral immunity) response investigation. But due to lack of our possibilities we investigated only humoral immunity by quantitative detection of immunoglobulins. Unfortunately we had no opportunity to investigate the cellular immunity.

Our study showed an absolute evidence of humoral immunity decrease among inactive carrier seafarers. Normal value of immunoglobulins is for IgG 700-1600 mg/dl and for IgA 70-400mg/dl. In HBsAg carrier seafarers IgG average level was lower than minimal normal value – 83.4±12.8 mg/dl, and IgA average level was - 55.3 ±12.8 mg/dl (Fig 2).

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In our cases, immunoglobulins (antibodies) concentration decrease means that antibody response to HBV (surface antigens-HBsAg) is low and as a result body can't release a virus. It is known that antibody response is also cellular immunity dependent process, which plays a critical role in viral releasing of body.

Conclusions

As a rule supressed immunity may result in inactive carrier state. According to some researches the number of inactive carriers is much higher than active diseased seafarers. We may suppose that the reason of high percentage of inactive carriers among seafarers is immunodepresion caused by special conditions of labor on board. It is well known that virus reactivation among inactive carriers in case of immunodepression takes place in 20-30% of cases. So, the virus reactivation among inactive carrier seafarers may occur, especially on vessels transporting hazardous goods.

It should be also noted that HBsAg inactive carriers have increasing epidemiological risk in closed circumstances as board of ship. Statistics show that the hepatitis B virus is up to 30 times more infectious than HIV/AIDS and as known inactive carriers remain infectious to others through parenteral or sexual transmission.

In order to reveal the true reason of such high percentage of inactive carriers among seafarers it is necessary to carry out additional research. It is also advisable to introduce and examine the effectiveness of HBV vaccination.

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ჰეპატიტ B ვირუსის გავრცელების სიხშირე მეზღვაურთა შორის და HBsAg-ის მტარებელ პირთა ჰუმორული მაჩვენებელი

ახვლედიანი ლ.[1], ჭყონია გ.[2], ნაგერვაძე მ.[1], დუმბაძე გ.[1]

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[2]საზღვაო სამედიცინო სერვისი

(მიღებულია10.09.2007)

რეზიუმე

გამოკვლეულია 1165 მეზღვაურის პერიფერიული სისხლი და ამათგან 6.5% აღმოჩნდა HBsAg-ის არააქტიური მტარებელი. ნაჩვენებია, რომ ამ პირებში დაქვეითებულია ჰუმორული იმუნიტეტი. HBsAg-ის არააქტიურ მტარებელთა ასეთი მაღალი პროცენტი მეზღვაურთა შორის სავარაუდოდ გამოწვეულია საერთო იმუნოსუპრესიით, რაც განპირობებულია გემზე მუშაობის პირობებით.

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Immunology

INDICES OF THE PHAGOCYTIC ACTIVITY OF PERIPHERAL BLOOD NEUTROPHILS ON THE BACKGROUND OF CURING THE SUPPURATIVE OTITIS WITH "UNIMAG"

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Abstract

Indices of phagocytic activity of peripheral blood neutrophils in the process of curing the suppurative otitis with preparation "UNIMAG" have been investigated. It was revealed that on the third day of treating the phagocytic activity of peripheral blood polynuclear cells increased, and on the seventh day its significant activation was observed. Both, phagocytic number (22.5±1.1) and number of neutrophiles with phagocytic activity (56.8±3.1) enhanced by this period compared with the control. Intensification of the phagocytic activity of peripheral blood neutrophils while curing the suppurative otitis with "UNIMAG" is presumably stipulated not only by the inhibition of pathogenic microorganisms, but also by the direct influence of "UNIMAG" composing magnetite nano-particles on neutrophils.

Key words: neutrophils, phagocytic activity, suppurative otitis, preraration "UNIMAG"

Introduction

Inhibition of macro- and micro-phages activity, together with the influence of pathogenic flora, is the reason for inflammatory diseases of soft tissues and low effectiveness of therapeutic measures. As a result, application of therapeutic agents of combined activity, responsible for affecting the pathogenic flora, and strengthening the functional activity of inflammatory cells, becomes obligatory [Mashiro et al., 2001; Throp et al., 2000].

Literature data of the last years provide evidence for curing the soft and bone tissue suppurative-septic inflammations by means of domestic industry preparation "UNIMAG" [Saralidze et al., 2005; Surguladze et al., 2006; Tskitishvili et al., 2004].

The drug "UNIMAG" is a suspension of magnetite nano-particles. It is registered as an antibacterial and anti-inflammatory preparation by the Georgian Pharmaceutical Agency, and is distinguished by its combined effect and is intended for curing the suppurative-septic processes of different localization, etiology and pathogenesis.

The aim of our study was to determine the phagocytic activity of peripheral blood neutrophils while curing the purulent otitis with the preparation "UNIMAG".

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Materials and Methods

In vivo experiments were done in two series on Vistar race, 3 month old, and 140-150g weight white male rats (20 rats in each series of experiments).

The experimental animals were infected with pathogenic microorganisms through the artificially punched hole in tympanic membrane, under anaesthetic. In experimental group of rats otitis was treated with "UNIMAG", while in control group – by the traditional methods of therapy.

The drug therapy was applied on the 3rd day after infection, on the background of evident suppurative inflammation. Therapeutic treatments were performed once per day.

For the purpose to study the influence of the drug "UNIMAG" on phagocytic activity of the peripheral blood neutrophils the leukocytic mass was separated from the fresh, on heparin obtained blood of animals. The phagocytic activity of peripheral blood neutrophils was determined by standard method. Phagocytic number (PN) and phagocytic index (PI – the amount of phagocytose-able active cells) were determined during the experiments.

The testing material was taken before the treatment with drug, and on the 3-rd and 7-th days after its application.

Obtained results were statistically analyzed using the computer program SPSS 12.0 for Windows.

Results and Discussion

According to experimental results it was revealed that the phagocytic activity of peripheral blood polynuclear cells in experimental animals was significantly lower ($P<0.05$), compared with intact rats.

Treatment with "UNIMAG" caused increasing of phagocytic activity of peripheral blood polynuclears (PN – 13.4±0.3; PI – 27.2±0.9) in experimental animals on the 3rd day of therapy and significant progress of these indices on the 7th day ($P<0.05$) of application was observed, compared with both, control and pre-curing data. In particular, both PN (22.5±1.1) and PI (56.8±3.1) increased compared with the control (Figures 1, 2).

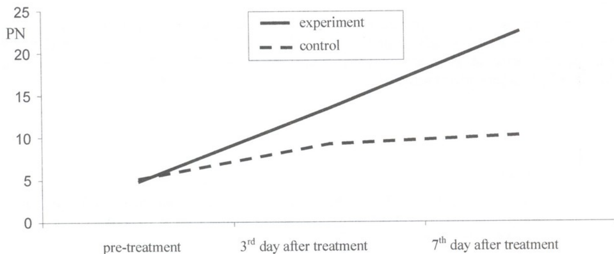

Fig. 1. Dynamics of phagocytic number of peripheral blood neutrophils on the background of treating with the preparation "UNIMAG"

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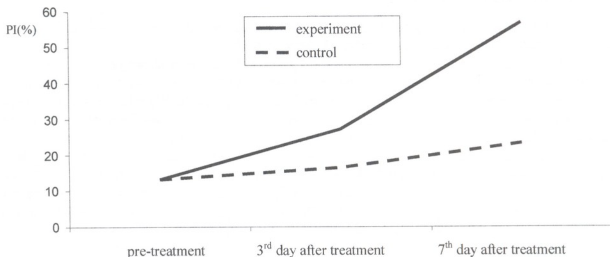

Fig. 2. Dynamics of active cells in peripheral blood neutrophils on the background of treating the suppurative otitis with the preparation "UNIMAG"

Stimulation of the phagocytic activity of cells under the effect of drug "UNIMAC" is of great importance in suppurative otitis therapy, since it is known that low efficiency of phagocytosis against the microflora may become a reason for hyper-activation of neutrophils metabolism, which from its side leads to deepening of destructive processes.

No significant changes of phagocytic activity of peripheral blood polynuclears were observed ($P>0.05$) three days after the application of traditional complex therapy of suppurative otitis. Seven days after treatment the PI increased reasonably compared with pre-curing and 3-days treatment results. It must be mentioned that 7 days after starting the therapy PI and PN data of control group were statistically true lower, compared with 3rd and 7th days experimental results ($P<0.05$).

We suppose that intensification of phagocytic activity of peripheral blood neutrophils on the background of treatment of suppurative otitis with preparation "UNIMAG" may be caused by the direct influence of magnetite nano-particles composing "UNIMAG", together with the inhibition of pathogenic microorganisms.

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პერიფერიული სისხლის ნეიტროფილების ფაგოციტური აქტივობის მაჩვენებლები ჩირქოვანი ოტიტების "უნიმაგით" მკურნალობის ფონზე ექსპერიმენტში

გორდელაძე გ., ხეჩინაშვილი ს., ცქიტიშვილი თ., სურგულაძე ბ.

სამეცნიერო კვლევითი ლაბორატორია "მაგნიტური სითხეები მედიცინასა და ბიოლოგიაში" (შპს "ატტ")

(მიღებულია 07.12.2006)

რეზიუმე

შესწავლილია პრეპარატ "უნიმაგით" ჩირქოვანი ოტიტების მკურნალობისას პერიფერიული სისხლის ნეიტროფილების ფაგოციტური აქტივობის მაჩვენებლები. დადგენილია, რომ ამ პრეპარატით მკურნალობის მე-3 დღეს ცხოველთა პერიფერიული სისხლის პოლინუკლეარების ფაგოციტური აქტივობა მატულობს, 7 დღის შემდეგ კი – მნიშვნელოვნად ძლიერდება. კერძოდ, ამ პერიოდისათვის მატულობს როგორც ფაგოციტური რიცხვი, ასევე ფაგოციტირების უნარის მქონე ნეიტროფილების რაოდენობაც, საკონტროლო მაჩვენებლებთან შედარებით. პერიფერიული სისხლის ნეიტროფილების ფაგოციტური აქტივობის გაძლიერება ჩირქოვანი ოტიტების პრეპარატ "უნიმაგით" მკურნალობისას სავარაუდოდ განპირობებულია არა მხოლოდ პათოგენური მიკროორგანიზმების დათრგუნვით, არამედ "უნიმაგის" შემადგენელი მაგნეტიტის ნანო-ნაწილაკების ნეიტროფილებზე პირდაპირი ზემოქმედებით.

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MICROSCOPIC FUNGI SPREAD IN SOLIS OF SHIDA KARTLI AND SAMEGRELO

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Abstract

In search of new active producers of enzymes 72 species of microscopic fungi have been isolated from two different soil-climatic ecologic niches – moderately dry subtropic (Shida Kartli region) and humid subtropic (Samegrelo region). The 5 genera belonging to the classes Ascomycetes, Zygomycetes and Deuteromycetes are identified. Comparison is made between the microflora of humid subtropical and moderately dry soil-climatic zones. Frequency of occurrence in soil is determined for each genus and prevailing genera are established. Dry soil-climatic zone is found to be distinguished by the abundance and diversity of microscopic fungi as compared with humid subtropics.

Key words: microscopic fungi, enzyme producer, soil-climatic zone.

Introduction

Collections of cultures of microorganisms are important for the development of certain fields of biology. Necessity of creation of new microbial collections was conditioned by the recent rapid development of molecular biology, molecular genetics and genetic engineering. Such collections allow revealing of new strains of industrial importance, which can be used in wide scale for obtaining expensive products of microbial origin. Determination of strains producing stable enzymes seems to be especially important in this respect. Enzyme-based biotechnologies can be assessed as reliable, low-waste, healthy and comparatively ecologically low-risky methods. Thus selection of microorganisms growing in different habitats and evaluation of their resistance to different critical conditions seems to be urgent at present [Hunter et al., 1984].

Materials and Methods

A total of 10 averaged soil samples have been taken from moderately dry subtropic and humid subtropic zones of Georgia – Shida Kartli region and Samegrelo region [Fomin G., Fomin A., 2001]. In Shida Kartli region soil samples were taken at different depths from the orchards and wooded zone, and in Poti environs (Samegrelo region) from the coastal zone of Paliastomi Lake, the territory adjacent to the terminal and Maltakva coast.

In order to obtain a homogenous suspension the soil aggregates were dispersed and cells of microorganisms were subjected to the desorption and microcolonies separated into single cells

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[Zvyagintsev et al., 1980]. Soil samples treated in such way were sown according to the method of dilution [Waksman S., 1916].

Microorganisms were isolated on cultivation media of the following composition (g/l): 1. Czapek's modified medium - Starch 20.0, $NaNO_3$ - 9.1; KH_2PO_4 - 1.0; $MgSO_4x7H_2O$ - 0.5; KCl – 0.5; $FeSO_4$ - 0.02; agar-agar 20.0 (pH 5.5-6.0). 2. Czapek-Dox agar - Sucrose 30.0; $NaNO_3$ - 2.0; KH_2PO_4 - 1.0; $MgSO_4x7H_2O$ - 0.5; KCl - 0.5; $FeSO_4$ - 0.02; agar-agar 20.0 (pH 5.5-6.0). 3. Selective cultivation medium – microcrystalline cellulose 10.0; $NaNO_3$ - 3.0; KH_2PO_4 - 2.0; $MgSO_4x7H_2O$ – 0.5; Fe SO_4 - 0.02, yeast extract 10.0, agar-agar 20.0 (pH 5.5-6.0). 4. Czapek's acidified medium- glucose 10.0; $NaNO_3$ -9.0; KH_2PO_4 -1.0; $MgSO_4x7H_2O$ – 0.5; KCl -0.5; $FeSO_4$ - 0.02, agar-agar-20.0. 5. Universal cultivation medium - Universal Beer Agar 0.5 *l* of 7 Balling beer malt, 0.5 *l* of tap water, agar-agar 20.0 (pH 5.5-6.0).

Cultivation medium was sterilized at 0.6 atm. for 45 minutes. Microscopic fungi were incubated in thermostate at 30°C. Description and evaluation of samples was made on the 3rd day from sowing and was monitored during 10 days.

In order to obtain pure cultures sowing on the universal cultivation medium of individual colonies developed on Petri dishes was started on the 5th day of incubation. Purified colonies were kept in the fridge at +3°C.

Frequency of occurrence of individual genera was calculated according to the formula: frequency of occurrence of individual genus = number of samples, where the given genus is detected/total number of genera.

While identifying microscopic fungi isolated from the soil up to genus cultural-morphological characteristics and the peculiarities of structure of reproduction organs were taken into consideration [Kreisel, 1969]. Cultural-morphological properties were studied using the method of microscopy. In most cases the preparations of different types were made, which were studied by dry optical system. Identification of fungi was made using the Identifcation Books (keys) by Podoplichko, Bilai, Litvinov and Maloch.

With the aim of searching for mcroscopic fungi two different regions within 20 soil-climatic zones of Georgia have been chosen – Shida Kartli – moderately dry subtropical zone and Samegrelo region – humid subtropical zone. The villages of Shida Kartli – Shindisi and Akhaldaba are situated in the zone of plane-foothills and are characterized with moderately cold winter and hot summer. This region represents steppe with brometo-stipetum and astragaletum, with alluvial carbonate and non-carbonate soils. Paliastomi Lake and village Maltakva belong to swampy podzol soils of Colchis lowland, which is characterized by alluvial carbonate and non-carbonate soils, humid climate, warm winter and hot summer.

Results and Discussion

Microscopic fungi were isolated on nutrition media of different composition. Universal agarized cultivation medium was distinguished by rich microflora. Almost all isolated fungi grew on this medium. Comparatively less microflora developed on acidified and modified Czapek's media. Microorganisms grew poorly on selective and Czapek-Dox media.

Determination of colony forming unit (CFU) has shown that soils of Shida Kartli region are characterized by richness of microscopic fungi (Table 1).

Table 1. Number of colonies of microscopic fungi isolated form soils of Shida Kartli and Samegrelo

Place of sampling	Number of colonies per 1 g of dry soil (CFU) (arithmetical mean)
Samegrelo region	$0.8x10^2$
Shida Kartli region	$3.5x10^3$

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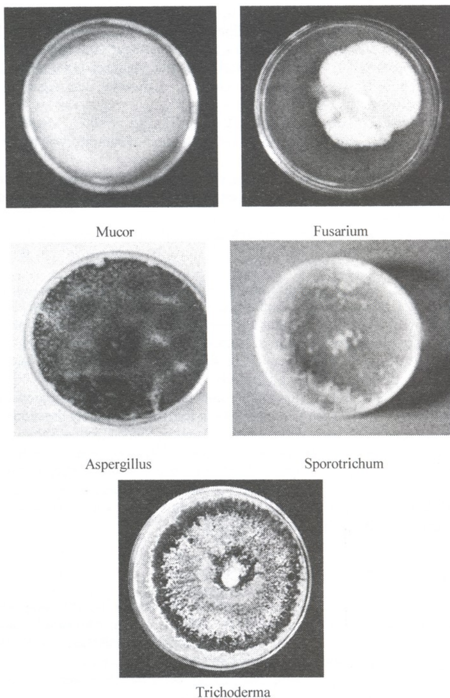
As a result of experiment 72 species of microscopic fungi have been isolated from the chosen soil-climatic zones – 46 from soils of Shida Kartli and 26 from soils of Samegrelo region. The 5 genera of microscopic fungi – Aspergillus, Fusarium, Trichoderma, Mucor and Sporotrichum belonging to classes **Ascomycetes**, **Zygomycetes** and **Deuteromycetes** are identified (Fig. 1).



Mucor Fusarium

Aspergillus Sporotrichum

Trichoderma

Fig.1. Microscopic fungi belonging to different genera

The obtained results are in conformity with data available in scientific literature, suggesting that the genera Aspergillus, Trichoderma and Mucir are mainly found in soils.

Figures 2 and 3 illustrate the composition and frequency of occurrence of microscopic fungi spread in soils of Shida kartli and Samegrelo region. Microflira of dry subtropical zone turned out to be more diverse than that of humid subtropical zone.

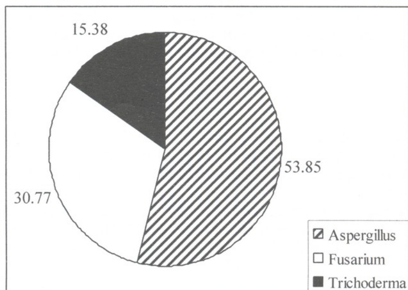


Fig. 2. Frequency of occurrence of microscopic fungi in soils of dry subtropical zone (Shida Kartli region)

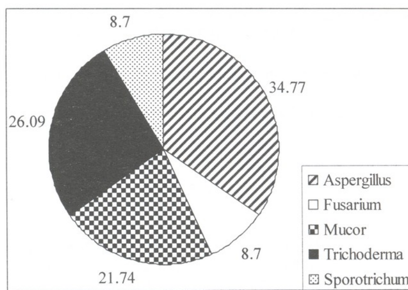


Fig. 3. Frequency of occurrence of microscopic fungi in soils of humid subtropical zone (Samegrelo region)

Among 5 identified genera Mucor and Sporotrichum are not found in Samegrelo soils (Fig. 2, 3). Microflora of climatic zones differed in dominant genera as well.

The genus Aspergillus is an absolute dominant in humid subtropical zone, while the genus Fusarium is a dominant of the first rank. Absolute dominant has not been revealed in moderately dry subtropical zone. Here the genera Aspergillus and Trichoderma are the dominants of the firs rank.

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Conclusions

Thus on the basis of carried out experiments collections of microscopic fungi are created for two different soil-climatic zones of Georgia - moderately dry subtropical and humid subtropical zones. The collection requires further investigation in order to establish physiological and biochemical characteristics of individual cultures. This will enable us to reveal new, active producers of enzymes, which is of great practical importance for biotechnological industry.

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შიდა ქართლისა და სამეგრელოს ნიადაგებში გავრცელებული მიკროსკოპული სოკოები

ქუთათელაძე ლ., ზაქარიაშვილი ნ., ალექსიძე თ. ჯობავა მ.,

დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 25.07.2007)

რეზიუმე

სხვადასხვა ფერმენტების ახალი, აქტიური პროდუცენტების ძიების მიზნით საქართველოს ორი განსხვავებული ეკოლოგიური ნიშიდან – ზომიერად მშრალი სუბტროპიკული (შიდა ქართლის რეგიონი) და ნოტიო სუბტროპიკული (სამეგრელოს რეგიონი) ნიადაგობრივ-კლიმატური ზონებიდან გამოყოფილია 72 განსხვავებული სახეობის მიკროსკოპული სოკო. იდენტიფიცირებულია 5 გვარი, რომლებიც მიეკუთვნება კლასებს Ascomycetes, Zygomycetes და Deuteromycetes. შედარებულია ნოტიო სუბტროპიკული და ზომიერად მშრალი სუბტროპიკული ნიადაგობრივ-კლიმატური ზონების მიკროფლორა, განსაზღვრულია ნიადაგებში ცალკეული გვარის შეხვედრის სიხშირე და გამოვლენილია დომინანტი გვარები. დადგენილია, რომ მიკროსკოპული სოკოების უფრო მაღალი სიუხვითა და მრავალფეროვნებით ხასიათდება ზომიერად მშრალი სუბტროპიკული ნიადაგობრივ-კლიმატური ზონა.

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ISOLATION AND SELECTION OF THE TOLUENE DEGRADING BACTERIA

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Abstract

Bacteria capable to apply the toluene as a sole source of carbon and energy have been isolated from the soil adjacent to one of the boreholes in significant for Georgia oil deposits (Sartichala). As a result of multi-step screening the strains with high ability of toluene degradation under conditional numbers TT306 and TT304, utilizing toluene in the amounts of 36% and 39%, respectively, have been selected.

Key words: Sartichala oil deposit, soil samples, colony forming unit, residual toluene.

Introduction

Oil, oil containing certain constituents and oil products are the most widespread among environmental contaminants. During oil production oil-contaminated soils are generated. Oil constituent toxic hydrocarbons accumulating in animal and plant tissues have a deleterious effect on vitality of many organisms [Alvarez et al., 1991]. Being inserted into the food chain, they threaten human health. Especially problematic is environmental contamination with BTEX (benzene, toluene, ethylbenzene and xylene), since they are characterized by relatively high water solubility (0.053 g/100 ml of water at 20°C) and toxicity [Nicholson et al., 2004]. The limited permissible dose of toluene (methylbenzene – $C_6H_5CH_3$), one of the constituents of BTEX is 0.5 mg/l. It has weak narcotic action and influences the blood-vascular and nervous systems as well. Therefore, selection of strains capable to degrade toluene and elaboration of effective methods for refinement of soil and water contaminated with this compound is of great importance.

The goal of the present work is to isolate bacteria with toluene biodegradative capability from oil-contaminated territories of Georgia.

Materials and Methods

To isolate toluene-degrading bacteria soil samples have been taken from the area adjacent to one of the boreholes of Sartichala oil deposit.

Standard methods were used to take averaged samples of soil, to prepare soil suspension and for quantitative analysis of microorganisms [Pramer et al., 1972; Zvjagintsev, 1973; Anikiev et al., 1983]. Beef-extract agar and glucose-containing Czapek's medium (g/l: glucose – 20, $NaNO_3$ –

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2, K_2HPO_4 – 1, $MgSO_4 \times 7H_2O$ – 0.5, KCl – 0.5, $FeSO_4 \times 7H_2O$ – 0.01, agar – 15, pH 7.0) were applied to isolate bacteria from soil suspension. Microorganisms were incubated in thermostat at 30°C for 10 days.

In order to isolate toluene-degrading bacteria so-called culture enrichment method was applied on selective substrate (toluene was used as a sole source of energy and carbon).

The amount of colony forming unit (CFU) was evaluated in per g of dry soil.

For screening freshly isolated bacteria with toluene biodegrading potential and microorganisms kept in the Durmishidze Institute of Biochemistry and Biotechnology, cultures were grown on agar media to which 2% (vol.) of toluene was added as a sole source of carbon and energy. Bacteria were incubated on solid nutrient media in thermostat at 30°C during 7 days and in liquid ones – in 750-ml flasks, on a rotary shaker (180 rev/min) at 28-30°C, during 2 weeks.

The growth intensity of bacteria on toluene-containing solid media was estimated visually by 5-point system (– - no growth, + - trace, 2+ - poor growth, 3+ - average growth, 4+- intensive growth).

The inoculation of liquid medium (50 ml) was conducted by 10% bacterial suspension at exponential growth. The following media were used as controls: (a) the mineral base of Czapek's medium containing toluene without microbial inoculates and (b) the medium without toluene (in the presence of glucose) by adding of microbial inoculates.

To determine growth intensity of bacteria in liquid nutrient medium by weight method, bacterial suspension was centrifuged at 6000 *g* during 15 minutes.

The amount of residual toluene was determined after its trice extraction by hexane from the cultural solution by spectrophotometric method at wavelength of 253 nm (Specord UV-VIS M-400, Germany).

Results and Discussion

To isolate toluene-degrading bacteria soil samples have been taken from the area adjacent to one of the boreholes of Sartichala oil deposit, particularly:

- freshly contaminated soil in the depth of 15 cm;
- freshly contaminated soil in the depth of 25 cm;
- long-standing contaminated soil in the depth of 15 cm;
- long-standing contaminated soil in the depth of 25 cm;
- long-standing contaminated soil in the depth of 50 cm.

The same type of soil, without oil contamination was used as a control.

Chemoorganotrophic species degrading organic pollutants, capable of applying natural compounds and xenobiotics as sources of energy and electron donors to obtain energy, are prevalent in the contaminated zone [Van Hamme et al., 2003].

On the base of experimental data, it has been revealed that oil contamination causes activation of oil-degrading microflora at the expense of creating favourable conditions for propagation of hydrocarbon reducing bacteria. For instance, number of bacteria in oil-contaminated samples of soil equals to 28×10^6 - 45×10^6 and in control is one order less.

Microbiota of soil samples are characterised by bacterial biodiversity (Fig. 1). Colonies of various colour, shape and size occur there. Dominant bacteria of contaminated soils belong to the following genera: *Pseudomonas, Rhodococcus*, *Nocardia*, *Mycobacterium* and *Bacillus,* which express remarkable morphological diversity.

It is known that bacteria from the above-mentioned genera are distinguished by various high metabolic activities, and the ability to synthesize surfactants [Houghton, Shanley, 1994; Kim et al., 2004[.

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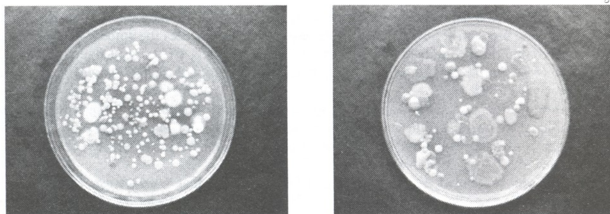

Fig. 1. Microbial consistence of soil from the area adjacent to Sartichala borehole.

The amount of colony forming units (CFU) on toluene-containing media was $n \times 10^3$, which is lower by three orders than that of bacteria grown on the same mineral medium in the presence of glucose.

32 strains growing on toluene containing medium were obtained after manifold inoculation and cleaning of freshly isolated bacteria from the mentioned soil samples. To determine toluene utilising ability and intensity the screening of freshly isolated bacteria and collection strains of Durmishidze Institute of Biochemistry and Biotechnology was conducted.

Initial screening was carried out on agar media, in which toluene (2%) was the sole source of carbon and energy. Toluene degrading capability was estimated visually by 5-point system („–" – no growth and „4+" – intensive growth).

Eight strains, growth intensity of which were estimated as intensive (4+) on toluene-containing solid medium have been selected for further experiments.

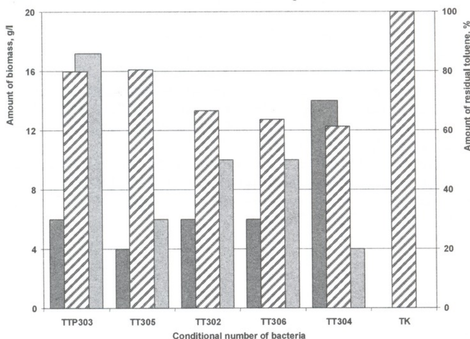

Fig. 1. The growth intensity of bacteria and toluene utilisation under the conditions of applying toluene as a sole source of carbon and energy: ■–Biomass in the presence of toluene, g/l; ■–Biomass in the presence of glucose, g/l; ▨ Amount of residual toluene, %.

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Among the test strains five bacterial cultures were characterized by growing in liquid toluene-containing medium (Fig. 1). In control (energy source – glucose) the amount of biomass in bacteria under conditional numbers TTP303 and TT305 were 35%, 67%, respectively, in TT302 and TT306 - 60%; and in case of toluene application as a sole growth factor, the index exceeded control 3-times in TT304.

Determination of residual toluene by spectrophotometric method has shown that strains under conditional numbers TT306 and TT304 displayed the highest toluene degrading potential (Fig. 1). After two-week incubation in the liquid medium the amount of utilised toluene by these strains equalled to 37% and 39%, respectively.

Based on conducted experiments might be concluded that consistence, structure and parameters of soil microflora alter in case of oil contamination. Bacteria with high toluene degrading potential, which are able to use toluene as a sole source of carbon and energy have been isolated from the area adjacent to borehole of Sartichala oil deposit, and selected via multi-step screening.

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ტოლუოლის დეგრადაციის უნარის მქონე ბაქტერიების გამოყოფა და შერჩევა

საჩანელი თ., ამირანაშვილი ლ., გაგელიძე ნ., ჯიმშელეიშვილი თ., თინიკაშვილი ლ., ვარსიმაშვილი ხ.

დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 16.07.2007)

რეზიუმე

საქართველოს ერთ-ერთი მნიშვნელოვანი, სართიჭალის ნავთობის საბადოს ჭაბურღილის მიმდებარე ტერიტორიის ნიადაგებიდან გამოყოფილია ბაქტერიები, რომელთაც შეუძლიათ ტოლუოლის გამოყენება ნახშირბადისა და ენერგიის ერთადერთ წყაროდ. მრავალსაფეხურებრივი სკრინინგის შედეგად შერჩეულია ტოლუოლის მაღალი დეგრადაციის უნარის მქონე შტამები პირობითი ნომრებით TT306 და TT304, რომელთა მიერ უტილიზებული ტოლუოლის რაოდენობა შეადგენს 36% და 39%-ს, შესაბამისად.

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Microbiology

PRODUCING OF PROTEIN-RICH BIOMASS BY DIRECT CULTIVATION OF MICROMYCETES ON LIGNOCELLULOSE-CONTAINING SUBSTRATE AND ENZYMATIC HYDROLYSIS OF CELLULOSE-CONTAINING RAW MATERIAL

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Abstract

The ability of thermophilic micromycetes *Aspergillus versicolor, Aspergillus wentii, Sporotrichum pulverulentum* to synthesize protein-rich non-toxic and non-pathogenic biomass while direct cultivation on a lignocellulosic waste (residuals of tea industry and one-year cuttings of vine) has been demonstrated. Obtained protein-rich biomass may be used as a food supplement in livestock farming. To obtain various sugar syrups and glucose hydrolysis of lignocellulosic substrates treated previously with industrial preparations of selected strains has been studied.

Key words: bioconversion, cellulase, lignocellulosic waste

Introduction

Protein-rich biomass, sugars and other products may be obtained by means of non-traditional microbial and enzymatic processing of agricultural lignocellulosic waste, which may be further used for household, chemical, medical and other purposes.

Many microbial strains are able to perform a conversion of lignocellulosic substrates [Chang and holtzapple, 2000; Jacobsen and Wymann, 2000], but the problem is detection of non-pathogenic and non-toxic strains producing stable and active cellulatic enzymes. Selection of more perspective substrates for a particular region is also important. In Georgia wastes of tea-plant and vine industry are more important. These residuals do not contain toxic substances, permanently refresh and are quite rich of cellulose (30-32%). Accordingly, this mass enriched with mineral substances represents a favorable substrate for receiving a protein-rich biomass via bioconversion performed with actinomycetes.

Thermophilic micromycetes, producers of active cellulases, are especially popular from the point of view of bioconversion of cellulose containing substrate. These micromycetes effectively utilize cellulose at high temperature range (temperature of pasteurization), which minimizes possibility of reactors pollution.

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Materials and Methods

Active producers of cellulases have been selected among the thermophilic strains of micromycetes collection of Durmishidze Institute of Biochemistry and Biotechnology: *Aspergillus versicolor, A. wentii* (optimal temperature at 60°C), *Sporotrichum pulverulentum* (optimal temperature 55°C). For the purpose to obtain the protein-rich biomass (residuals of tea and vine) the selected cultures were grown by means of submerged cultivation on the following nutrient medium: 10% 0f $NaNO_3$- 3g; KH_2PO_4 – 2g; $MgSO_4 \cdot 7H_2O$ – 0.5g per liter. pH of the medium – 4.5. Incubation took place on a thermostatic shaker at 40°C, 200rot/min for 96h. Tea and vine waste was preliminarily dried at 60°C and ground (500-600μm). Suspension of 10-days old conidia was used as a sowing material. After cultivation the obtained biomass was filtered, dried at 105°C and ground for analysis. Microcrystal cellulose (1%) was used as carbon source instead of waste for obtaining the active enzyme preparation under the submerged cultivation in a nutrient medium.

The technical preparations of cellulases were received by means of sedimentation with 4 volumes of ethanol from the cultural solutions under cool conditions. Obtained sediment was subjected to dialysis and lyophilized.

The biochemical analysis of the biomass was performed according to Stepurina (1984). Amino acids were determined on an amino analyzer, reducing sugars – by means of Somojy-Nelson's modified method [Ghose, 1987]; content of glucose [Sherbukhin et al., 1970], toxicity and pathogenicity [Spesivtseva, 1971] were also investigated. Substrates were preliminarily treated using the oxidative delignification method [Brownell and saddler, 1987].

Results and Discussion

To investigate the toxicity of the selected micromycetes specially prepared sample was implicated twice with 24h interval on a shaved skin of a rabbit and the skin reaction was studied. To study the pathogenicity different doses of suspension of the selected micromycetes diluted with a physiological solution were injected intravenous.

Clinical observations and morbid anatomical investigations were performed to analyze the experimental results. No pathogenic changes were mentioned in both cases, indicating that both biomass and enzymes produced by the selected micromycetes were not toxic and pathogenic and may be successfully used for a various industrial and agricultural purposes. The results of biochemical analysis of micromycetes cultivated on tea industry residuals and one-year cuttings of vine are demonstrated in Table1.

Table 1. The chemical composition of biomass obtained by means of cultivation of thermophilic micromycetes *Aspergillus versicolor, A. wentii, Spirotrichum pulverulentum* on tea and vine waste

Biomass composition, %	*Sp.pulverulentum*	*A.versicolor*	*A.wentii*	*Sp.pulverulentum*	*A.versicolor*	*A.wentii*
Water	2.27	2.1	1.69	2.1	2.3	1.9
Calcium	0.2	0.3	0.5	0.4	0.2	0.15
Phosphorus	0.26	0.7	0.45	0.49	0.39	0.43
Carotene, μg/g	15.0	10.2	15.0	0	0	0
Fat	7.88	4.3	5.7	6.7	3.0	4.2
Cellulose	24.05	24.2	24.06	18.7	19.0	19.0
Protein	24.6	26.0	27.8	22.6	20.0	20.0
Non-nitrogen extractable substances	36.11	35.0	35.2	31.2	32.1	30.5

From the obtained results it is clear that protein content of a biomass varied insignificantly. Amount of proteins in the biomass obtained from the waste of tea industry was in some extent higher compared with the biomass received from the fermentation of vine cuttings.

Moreover, the biomass obtained from tea residuals contained significant amount of a growth factor - carotene. Content of calcium, phosphorus, fats and non-nitrogen extractable substrates in biomass was at a level of demanded norms.

For appreciation the nutrition properties of the biomass obtained from lignocellulosic waste the amino acid composition of the biomass was studied (Table 2).

According to the analysis microbial protein contained all irreplaceable amino acids and its nutritional value was high. Thus, direct cultivation of selected micromycetes on tea and vine waste produced protein-rich, non-pathogenic and non-toxic biomass, which may be used as a food supplements in livestock farming.

On the other hand fermentation of cellulose-containing waste is perspective for obtaining different sugar syrups and glucose. Thus on the next step of investigations hydrolysis of tea and vine waste was studied using the high-active technical preparations of cellulases. It is known that cellulose in plants exists in the form of lingo-hydrocarbon complex, which complicates cellulose reactivity for the enzyme. Hence, the preliminarily treatment of the substrate is necessary. Cellulose-rich samples (70-75%) are obtained by means of an oxidative delignification.

Table 2. Content of amino acids in the biomass obtained via cultivation of thermophilic micromycetes *Aspergillus versicolor, A. wentii, Spirotrichum pulverulentum* on tea and vine waste

Amount of amino acids on 100g of dry biomass, g	*Sporotrichum pulverulentum*	*Aspergillus versicolorA*	*Aspergillus wentiiA*	*Sporotrichum pulverulentum*	*Aspergillus versicolor*	*Aspergillus wentii*
Lysine	0.58	1.5	1.3	0.55	1.5	1.4
Arginine	0.87	1.2	1.1	0.86	1.0	0.6
Aspartic acid	1.24	1.7	2.7	1.58	1.0	1.2
Histidine	0.37	1.7	2.0	0.32	1.7	2.4
Threonine	0.63	0.9	1.0	0.76	0.9	0.8
Serine	0.58	1.1	1.5	0.68	1.1	0.8
Proline	-	0.8	0.7	-	-	-
Glycine	0.74	1.0	1.5	0.83	1.0	1.2
Alanine	0.77	1.2	2.4	0.98	0.9	0.9
Cysteine	0.17	0.18	0.2	0.20	0.18	0.18
Valine	0.89	1.1	1.1	1.13	1.1	0.9
Methionine	0.40	1.2	1.0	0.47	1.0	1.1
Izoleicini	0.69	1.5	2.5	0.82	1.1	1.1
Leucine	0.15	1.4	2.8	1.46	2.0	2.3
Tyrosine	0.38	0.5	0.5	0.51	1.1	1.3
Phenylalanine	0.81	0.8	1.0	1.02	1.1	1.3
Glutamic acid	1.76	2.0	2.8	1.08	2.0	1.5

Table 3. Action of endoglucanases preparations from thermophilic fungi on pretreated substrates

Substrates	Tea wastes	Wine wastes	Conversion %
A.versicolor	72	48	reducible sugars
	78	50	glucose
A.wentii	70	44	reducible sugars
	74	46	glucose
Sp.pulverulentum	60	40	reducible sugars
	64	43	glucose

Residuals of tea and vine industry were previously treated before hydrolysis. Process of hydrolysis was performed in a thermostatic (65°C) permanent-type reactor. 24h and 48h after

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hydrolysis content of reducing sugars and glucose was determined in the incubation area. According to these data the efficiency of the hydrolysis was assessed. It must be mentioned that 24h after hydrolysis no further increment of sugars was mentioned, because of this in table 3 the results of 24h conversion are demonstrated.

From the obtained results it is clear that the extent of tea and vine waste hydrolysis using the technical preparations of cellulases produced from the selected micromycetes is quite high.

Besides, the most part of the reducing sugars was hydrolyzed till glucose. Thus, producing of sugar syrups and crystal glucose by fermentation of cellulose-containing substrates is realistic.

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ლიგნო-ცელულაზურ სუბსტრატებზე მიკრომიცეტების პირდაპირი კულტივირებით ცილით მდიდარი ბიომასის მიღება და ცელულოზაშემცველი ნედლეულის ფერმენტული ჰიდროლიზი.

ურუშაძე თ., ხვედელიძე რ., ჩხარტიშვილი დ., ბურდული თ., იაშვილი თ.

ს. დურმიშიძის სახ. ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი

(მიღებულია 14.05.2007)

რეზიუმე

ნაჩვენებია თერმოფილური მიკრომიცეტების *Aspergillus versicolor, Aspergillus wentii, Sporotrichum pulerulentum* უნარი ლიგნო-ცელულაზურ ნარჩენებზე (ჩაის წარმოების ნარჩენები, ვაზის ერთწლიანი ანასხლავი) პირდაპირი კულტივირებისას წარმოქმნან ცილით მდიდარი, არატოქსიკური, არაპათოგენური ბიომასა, რომელიც შეიძლება გამოყენებულ იქნეს მეცხოველეობაში კვებით დანამატად. შესწავლილია შერჩეული შტამების ცელულაზების ტექნიკური პრეპარატების მოქმედებით წინასწარ დამუშავებული ლიგნოცელულაზური სუბსტრატების ჰიდროლიზი სხვადასხვა შაქრების სიროფების, გლუკოზის მიღების მიზნით.

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Paleobiology

THE PALEOBOTANICAL CHARACTERISTIC OF KIMMERIAN DEPOSITS OF WESTERN GEORGIA

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Abstract

The full list of Kimmerian flora of Western Georgia is given. The presumable reasons of differences between systematic composition of macrofloras and palynological complexes are considered. The palynological material is analyzed by landscape-phytocenological method. In development of climate and vegetation 9 stages and 14 substages are distinguished. They can be considered as the base of detail subdivision of Kimmerian deposits of Western Georgia on small stratigraphycal unites - palynozones.

Key words: Kimmerian Age, macroremains, palynological complexes, vegetation, flora, climate.

Introduction

After the works devoted to paleobotanical study of Kimmerian deposits passed nearly half of century. Macrofossils from the localities of Duabi and Gulripshi were learned by Kolakovsky [Kolakovsky, 1956; 1958; Kolakovsky, Shakryl, 1978], palynocomplexes - by Mchedlishvili (1963). Now these data can't be compared with new materials from other stages of Neogene, which palynological complexes were interpreted by landscape-phytocenological method [Shatilova et al., 2003; 2005]. So, the necessity of new researches of Kimmerian deposits by the same method arose. The first results of our investigation were published in 2002 [Shatilova et al., 2002]. During our work we used the stratigraphycal scheme of Taktakishvili (1984), who divided the Kimmerian stage of Western Georgia into two horizons: Azovian (Kmr 1) and Kamishburunian (Kmr 2).

Material and Methods

The present work is based on the rich palynological material from 10 outcrops of Kimmerian deposits distributed on the whole territory of Western Georgia (Guria, Abkhasia, Megrelia). For reconstruction of the climate of Kimmerian Age we use landscape-phytocenological method. It does not give exact valuation of climate parameters, but it allows to reconstruct the displacement of natural zone boundaries. In the base of method lies the idea about vegetational formation that makes it more effective for interpretation of the data of palynological analysis.

The landscape-phytocenological method is usually used for reproduction the climate of plains, where the climatic zones (tundra, forest, steppe) occupied the big territories [Borsenkova, 1992]. Here their changes in time by themselves were the indexes of fluctuation of climate. The more complicated situation is in mountain regions, where some forest belts with different climatic

conditions existed simultaneously on small territories, as it was in Colchis where the change of climate led seldom to radical change of landscape. The displacement of boundaries of mountain belts and penetration of the components of one forest formation on the territory of other took place. In connection with this we adapted the landscape-phytocenological method to the conditions of mountain area and used it for the reconstruction of climate of separate vegetational belts [Shatilova et al., 2004].

Results and Discussion

Below is given the full list of Kimmerian flora, composed by macroremains and palynological data (Table 1) and the table of number of separate taxa, determined by both methods (Table 2).

Tab. 1. The full list of taxons of Kimmerian flora, determined by great remains of plants (m) and by palynological data (p)

Class	Family	Species	m	p
Briopsida	Sphagnaceae	Sphagnum sp.		p
Lycopodiopsida	Lycopodiaceae	Lycopodium alpinum L.		p
		Lycopodium annotinum L.		p
		Lycopodium clavatum L.		p
		Lycopodium densum Sw.		p
		Lycopodium selago L.		p
		Lycopodium serratum Tunb.		p
		Lycopodium sp.		p
Isoetopsida	Selaginellaceae	Selaginella atrivirides Spring.		p
		Selaginella fusca N.Mtchedl.		p
		Selaginella pliocenica Dorof.	m	
		Selaginella selaginoides (L.) Link.		p
		Selaginella sp.		p
Equisetopsida	Equisetaceae	Equisetum sp.		p
Ophyoglossopsida	Ophyoglossaceae	Bothrychium sp.		p
		Ophyoglossum sp.		p
Polypodiopsida	Osmundaceae	Osmunda heeri Gaud.	m	
		Osmunda claytoniana L		p
		Osmunda regalis L.		p
		Todea sp.		p
	Schizaeaceae	Schizaea sp.		p
	Lygodiaceae	Lygodium digitatum Presl.		p
		Lygodium japonicum Sw.		p
		Lygodium sp.		p
	Pteridaceae	Cryptogramma crispa (L.)R.Br.		p
		Cryptogramma sp.		p
		Pteridacidites boerzoenyensis (Nagy) St., Sh. comb.nov.		p
		Pteridacidites dentatiformis Sh., St.		p
		Pteridacidites grandifoliiformis St., Sh.		p
		Pteridacidites guriensis Sh., St.		p
		Pteridacidites kimmeriensis Sh., St.		p
		Pteridacidites longifoliiformis Sh., St.		p
		Pteridacidites rarotuberculatum Sh., St.		p
		Pteridacidites remotifolioides Sh., St.		p

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Polypodiopsida	Pteridaceae	Pteridacidites spiniverrucatum St., Sh.		p
		Pteridacidites venustaeformis St., Sh.		p
		Pteridacidites verus (N.Mtchedl.) Sh., St. comb.nov.		p
		Pteridacidites vittatoides Sh., St.		p
	Parkeriaceae	Ceratopteris duabensis Kol.	m	
	Adiantaceae	Anogramma sp.		p
		Pityrogramma sp.		p
	Gleicheniaceae	Gleichenia sp.		p
	Polypodiaceae	Polypodium aureum L.		p
		Polypodium pliocenicum Ram.		p
		Polypodium serratum (Wild.) Futo		p
		Polypodium verrucatum Ram.		p
		Polypodium tuberculatum N.Mtchedl.		p
		Polypodium vulgare L.		p
		Polypodium sp.		p
		Verrucatosporites histiopteroides W.Kr.		p
		Pyrrosia sp.		p
	Hymenophyllaceae	Hymenophyllum rotundum N.Mtchedl.		p
	Dicksoniaceae	Dicksonia antarctica R.Br.		p
		Dicksonia reticulata Purc.		p
		Dicksonia unitotuberata Purc.		p
		Dicksonia aff. fibrosa Kol.		p
		Dicksonia sp.		p
	Cyatheaceae	Alsophylla sp.		p
		Cyathea sp.		p
	Aspleniaceae	Asplenium sp.		p
	Aspidiaceae	Athyrium sp.		p
		Cyclophorus sp.		p
		Cystopteris sp.		p
		Dryopteris sp.		p
		Gymnocarpium sp.		p
		Lastrea sp.	m	
		Struthiopteris filicastrum All.	m	
		Woodsia alpina (Bolton.) S.F.Gray		p
	Blechnaceae	Woodwardia sp.		p
	Salviniaceae	Azolla sp.		p
		Salvinia sp.		p
Ginkgoopsida	Ginkgoaceae	Ginkgo biloba L.		p
		Ginkgo occidentalis Samyl.	m	
Pinopsida	Podocarpaceae	Dacrydium sp.		p
		Podocarpus sp.	m	p
	Taxaceae	Taxus sp.		p
		Cephalotaxus sp.		p
	Araucariaceae	Araucaria sp.		p
	Pinaceae	Abies alba Mill.		p
		Abies cephalonica Loud.		p
		Abies ciliticaeformis N.Mtchedl.		p
		Abies nordmanniana (Stev.) Spach.		p
		Abies sp. cf. A. protofirma Tanai	m	
		Abies sp.	m	
		Cathaya abchasica Sveshn.	m	

Pinopsida	Pinaceae	Cathaya aff. argyrophylla C. et K.		p
		Cedrus atlantica Manetti		p
		Cedrus deodara Loud.		p
		Cedrus libani Laws.		p
		Cedrus sauerae N.Mtchedl.		p
		Keteleeria caucasica Ram.		p
		Picea complanataeformis N.Mtchedl.		p
		Picea minor N.Mtchedl.		p
		Picea orientalis L.		p
		Picea sp.	m	
		Pinus euxina Kol.	m	
		Pinus sp.	m	p
		Pseudolarix sp.		p
		Pseudotsuga sp.		p
		Tsuga aculeata Anan.		p
		Tsuga canadensis (L.) Carr.		p
		Tsuga diversifolia (Maxim.) Mast.		p
		Tsuga korenevae Mched.		p
		Tsuga meierii Mched.		p
		Tsuga inordinata Mched.		p
		Tsuga patens Downie		p
		Tsuga pattoniana Engelm.		p
		Tsuga tortuosa Mched.		p
		Tsuga shatilovae Mched.		p
		Tsuga sivakii Mched.		p
		Tsuga aff. blaringhemi Flous		p
	Sciadopityaceae	Sciadopitys sp.		p
	Taxodiaceae	Cryptomeria japonica Don	m	p
		Cunninghamia sp.		p
		Glyptostrobus europaeus (Brongn.) Heer	m	
		Glyptostrobus sp.		p
		Metasequoia sp.		p
		Sequoia sp.		p
		Taxodium sp.		p
		Taxodiaceae gen. indet.		p
	Cupressaceae	Libocedrus salicarnoides (Ung.) Heer	m	
		Juniperus sp.		p
		Cupressaceae gen. indet.		p
Ephedropsida	Ephedraceae	Ephedra sp.		p
Dicotyledoneae	Myricaceae	Comptonia sp.		p
		Myrica carolinensis Mill.		p
		Myrica palaeogala Pilar.	m	
		Myrica salicina Ung.	m	
		Myrica sp.		p
	Juglandaceae	Alfaroa sp.		p
		Carya aquatica (Michx.) Nutt.		p
		Carya cordiformis (Wangh.) C.Koch		p
		Carya ovata (Mill.) C. Koch		p
		Carya serraefolia (Goepp.) Krausel	m	
		Carya aff. glabra (Mill.) Sweet.		p
		Carya aff. pecan (Marh.) Engl.		p
		Carya sp.		p

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Dicotyledoneae	Juglandaceae	Cyclocarya aff. paliurus (Batalin) Jljinsk.		p
		Engelhardia sp.		p
		Platycarya sp.		p
		Pterocarya pterocarpa (Michx.) Kunth.	m	
		Pterocarya rhoifolia Sieb. et Zucc.		p
		Pterocarya stenoptera DC		p
		Pterocarya sp.		p
		Juglans cinerea L.	m	p
		Juglans regia L.		p
		Juglans zaisanica Jljinsk.	m	
	Salicaceae	Populus balsamoides Goepp.	m	
		Populus leucophylla Ung.	m	
		Populus populina (Brongn.) Knob.	m	
		Salix cinerea L.	m	
		Salix integra Goepp.	m	
		Salix varianus Goepp.	m	
		Salix sp.		p
	Betulaceae	Alnus angustifolia Kol.	m	
		Alnus hoernesi Stur	m	
		Alnus subcordata C.A.May	m	
		Alnus ducalis (Gaudin) Knob.	m	
		Alnus aff. barbata C.A.May	m	
		Alnus sp.	m	p
		Betula pubescens Ehrh.		p
		Betula sp.	m	p
		Carpinus betulus L.		p
		Carpinus caucasica Grossh.		p
		Carpinus grandis Ung.	m	
		Carpinus duabensis Dorof.	m	
		Carpinus orientalis Mill.		p
		Carpinus uniserrata (Kol.) Rat. et Kol.	m	
		Carpinus sp.		p
		Corylus sp.		p
		Ostrya angustifolia Andrean.	m	
		Ostrya sp.		p
	Fagaceae	Castanea atavia Ung.	m	
		Castanea sativa Mill.		p
		Castanopsis elisabethae Kol.	m	
		Castanopsis decheni (O.Web.) Kr. et Wld.	m	
		Castanopsis furcinervis (Rossm.) Kr. et Wld.	m	
		Castanopsis sp.		p
		Fagus attenuata Goepp.	m	
		Fagus orientalis Lipsky	m	p
		Fagus orientalis Lipsky var. palibini Jljinsk.	m	
		Quercus kodorica Kol.	m	
		Quercus neriifolia A.Br.	m	
		Quercus pseudocastanea Goepp.	m	
		Quercus sosnowsky Kol.	m	
		Quercus sp.		p
	Ulmaceae	Celtis sp.		p
		Ulmus carpinoides Goepp.	m	
		Ulmus foliacea Gilib.		p

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Dicotyledoneae	Ulmaceae	Ulmus laevis Pall.		p
		Ulmus longifolia Ung.	m	
		Ulmus paralaciniata Hu et Chaney	m	
		Ulmus sp.		p
		Zelkova carpinifolia (Pall.) Dipp.		p
		Zelkova zelkovifolia (Ung.) Buzek et Kotlaba	m	
		Zelkova sp.		p
	Eucommiaceae	Eucommia ulmoides Oliv.		p
	Moraceae	Cannabis sp.		p
		Ficus kolakovskyi Dorof. et Negru	m	
		Ficus sp.		p
		Morus alba L.		p
		Morus sp.		p
	Polygonaceae	Polygonum lapathifolium L.	m	
		Polygonum sp.		p
	Caryophyllaceae	Caryophyllaceae gen.indet.		p
	Chenopodiaceae	Chenopodiaceae gen.indet.		p
	Magnoliaceae	Liriodendron tulipifera L.		p
		Magnolia grandiflora L.		p
		Magnolia georgica Kol.	m	
		Magnolia denudata Desr.		p
		Magnolia kobus DC	m	
		Magnolia sp.	m	p
	Schizandraceae	Schizandra grossheimii Kol.	m	
	Lauraceae	Aniba longifolia Kol. et Schak.	m	
		Cinnamomum sp.		p
		Daphnogene buchii (Heer) Kol. et Schak.	m	
		Daphnogene marginatum (Kol. et Schak.) Kol. et Schak.	m	
		Daphnogene polymorpha (A.Br.) Etting.	m	
		Daphnogene sp.	m	
		Cinnamomophyllum cinnamomeum (Rossm.) Kol.	m	
		Cinnamomophyllum lanceolatum (Ung.) Kol.	m	
		Laurus sp.		p
		Laurophyllum abchasicum Kol. et Schak.	m	
		Laurophyllum duabense Kol. et Schak.	m	
		Laurophyllum primigenia (Ung.) Kol.	m	
		Laurophyllum simile Kol. et Schak.	m	
		Litsea magnifica Sap.	m	
		Persea braunii Heer	m	
		Persea cf.braunii Heer	m	
		Persea styracifolia (Weber) Kol.	m	
		Persea sp. aff. P. superta Sap.	m	
		Persea sp.		p
	Saxifragaceae	Saxifragaceae gen.indet.		p
	Ranunculaceae	Ranunculus reidii Szafer	m	
		Ranunculus sp.	m	p
	Menispermaceae	Menispermum sp.		p
		Sinomenium cantalense (E.M. Reid) Dorof.	m	
	Nymphaeaceae	Nelumbo sp.		p
		Nuphar luteum (L.) Smith		p

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Dicotyledoneae	Nymphaeaceae	Nymphaea sp.		p
	Actinidiaceae	Actinidia arguta (S. et Z.) Planch.	m	
		Actinidia faveolata C. et E.M. Reid	m	
	Theaceae	Eurya cf. japonica Thunb.	m	
		Schima wallichii (DC) Choisy	m	
	Hypericaceae	Hypericum sp.	m	
	Platanaceae	Platanus platanifolia (Ett.) Knob.	m	
		Platanus orientalis L.		p
	Hamamelidaceae	Hamamelis miomollis Hu et Chaney	m	
		Corylopsis sp.		p
		Fothergilla sp.		p
		Parrotia persica (DC) C.A.M.		p
		Sycopsis colchica Ram.		p
		Liquidambar europaea A.Br.	m	
		Liquidambar formosana Hance		p
		Liquidambar styraciflua L.		p
		Altingia sp.		p
	Rosaceae	Crataegus sp.	m	
		Rosa sp.		p
		Rubus sp.	m	
		Rosaceae gen. indet.		p
	Caesalpiniaceae	Cassiophyllum berenices (Ung.) Kr.	m	
	Fabaceae	Acacia sp.		p
	Geraniaceae	Geranium sp.		p
	Rutaceae	Phellodendron amurense Rupr.	m	p
	Simarubiaceae	Ailanthus sp.	m	
	Meliaceae	Cedrela sarmatica Kov.	m	
		Melia sp.		p
	Anacardiaceae	Pistacia sp.		p
		Rhus sp.		p
		Toxicodendron quercifolia (Michx.) Greene	m	
	Aceraceae	Acer integerrimum (Viv.) Mass.	m	
		Acer pseudomonospessulanum Ung.	m	
		Acer trilobatum (Sterb.) A.Br.	m	
		Acer sp.	m	p
	Sabiaceae	Meliosma caucasica Dorof.	m	
		Meliosma kimmerica Kol.	m	
	Aquifoliaceae	Ilex sp.		p
	Celastraceae	Euonymus sp.		p
	Staphyleaceae	Staphylea colchica Stev.		p
	Buxaceae	Buxus sempervirens L. foss. Englh. et Kinkelin	m	
	Rhamnaceae	Ceanotus ebuloides O.Weber	m	
		Ceanotus sp.	m	
		Rhamnus sp.		p
	Vitaceae	Ampelopsis europaea Dorof.	m	
		Ampelopsis ludwigii (A.Br.) Dorof.	m	
		Cissus sp. cf. C. adnata Plench.	m	
		Parthenocissus quinquefolia (L.) Planch.		p
		Vitis sp.		p
	Tiliaceae	Tilia cordata Mill.		p
		Tilia caucasica Rupr.		p

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Dicotyledoneae	Tiliaceae	Tilia platyphyllos Scop.		p
		Tilia aff. taqueti C. Schneid.		p
		Tilia sp.		p
	Sterculiaceae	Sterculia ramesiana Sap.	m	
		Sterculia sp.		p
	Thymellaceae	Daphne cf. pontica L.	m	
	Cucurbitaceae	Trichosanthus fragilis Reid.	m	
		Trichosanthus kodorica Kol.	m	
	Trapaceae	Trapa sp.	m	
	Myrtaceae	Myrtaceae gen.indet.		p
	Onagraceae	Chamaenerium sp.		p
		Ludwigia sp.		p
		Onagra sp.		p
	Alangiaceae	Alangium aff. kurzii Craib.		p
	Nyssaceae	Nyssa dissemonata (Ludw.) Kirchh.	m	
		Nyssa sylvatica L.		p
		Nyssa sp.		p
	Cornaceae	Bothrocaryum controversum (Hemsl.) Pojark.	m	
		Cornus sp.		p
		Thelycrania sanguinea (L.) Fourr.	m	
		Thelycrania lusatica Kirchh.	m	
	Araliaceae	Acanthopanax mirabilis (Kol.) comb. nov.	m	
		Acanthopanax kimmericus Kol.	m	
		Acanthopanax sp.		p
		Aralia cf. hispida Michx.	m	p
		Aralia cf. continentalis Katagawa	m	
		Aralia cf. cordata Thunb.	m	
		Aralia cf. hypoleuca Presl.	m	
		Aralia sp.		p
		Brassaiopsis sp. cf. B. glomeratula (Bl.) Regel.	m	
		Brassaiopsis sp.		p
		Dendropanax sp.		p
		Hedera multinervis Kol.	m	
		Hedera sp.		p
		Fatsia sp.		p
		Pentapanax simile Kol.	m	
		Araliaceae gen.indet.		p
	Apiaceae	Caucalis sp.		p
		Heracleocarpum protoponticum Kol.	m	
		Turgenia sp.		p
		Apiaceae gen. indet.		p
	Ericaceae	Rhododendron sp.		p
		Vaccinium raridentatum Sap.	m	
		Ericaceae gen. indet.		p
	Myrsinaceae	Rapanea kubanensis Pashkov	m	
	Sapotaceae	Sapotaceae gen.indet.		p
	Styracaceae	Halesia crassa (C. et E.M.Reid) Kirchh.	m	
		Halesia aff. diptera Ellis	m	
		Styrax raridentata Kol.	m	
		Styrax aff. japonica S. et L.	m	
	Symplocaceae	Symplocos abchasica Kol.	m	
		Symplocos antiqua Kol.	m	

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Dicotyledoneae	Symplocaceae	Symplocos paniculata Wall.		p
		Symplocos tinctoria (L.) L.' Her		p
		Symplocos sp.		p
	Apocynaceae	Apocynophyllum kimmericum Kol.	m	
	Oleaceae	Fraxinus sp.		p
	Caprifoliaceae	Lonicera sp.		p
		Viburnum lantana L.	m	
		Viburnum pliocenicum (Sap. et Mar.) Kol.	m	
		Viburnum tenuilobatum (Sap.) Kol.	m	
		Viburnum sp.		p
		Sambucus ebulus L.	m	
		Sambucus sp.	m	
	Lamiaceae	Ajuga antiqua C.et E.M.Reid		
		Lycopus sp.		
		Lamiaceae gen. indet.		
	Solanaceae	Solanum sp.	m	
	Dipsacaceae	Cephalaria sp.		p
		Dipsacus sp.		p
		Knautia sp.		p
		Scabiosa sp.		p
	Asteraceae	Artemisia sp.		p
		Asteraceae gen.indet.		p
Monocotyledoneae	Potamogetonaceae	Potamogeton pectinatus L.	m	
		Potamogeton sp.	m	
		Ruppia maritima L.	m	
	Poaceae	Poaceae gen. indet.		p
	Arecaceae	Arecaceae gen.indet.		p
	Sparganiaceae	Sparganium nanum Dorof.	m	
		Sparganium sp.		p
	Typhaceae	Typha latifolia L.		p
		Typha latissima A.Br.	m	
	Cyperaceae	Cladium aff. mariscus (L.) R.Br.	m	
		Cyperaceae gen. indet.		p

Tab. 2. Number of taxons in Kimmerian flora of Western Georgia determined by macroremains of plants (m) and by palynology (p).

Systematic Units	The Composition of Flora				Cryptogamous				Gymnosperms				Angiosperms			
	The Whole	m	p	The Common Taxa	The Whole	m	p	The Common Taxa	The Whole	m	p	The Common Taxa	The Whole	m	p	The Common Taxa
Form (Species)	375	147	241	13	71	5	66	0	53	11	45	3	251	131	130	10
Genus	189	91	142	44	35	5	32	2	25	9	24	8	129	77	86	34
Family	98	58	81	41	20	4	19	3	9	5	9	5	69	49	53	33
Class	11	6	11	6	6	2	6	2	3	2	3	2	2	2	2	2

The analysis of paleobotanical material revealed the definite differences between macrofloras and palynocomplexes. There are many reasons of this phenomenon, but we want to touch only four of them.

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The first reason is of systematical character. The researchers of macroremains of Kimmerian flora using the modern classification for genera and families distinguished the new fossil species. As for the palynological investigations, during our work we try to approach fossil grains to modern species.

The second reason is the differences in preservation of fossil material. The spores and pollen grains of gymnosperms and grasses preserved better than their macroremains. At the same time the pollen of some evergreen plants are seldom seen in deposits, which leathery leaves fossilized much better than pollen, especially the pollen grains of *Lauraceae*, which in phytolandscape of Kimmerian still took a big part [Kolakovsky, Shakryl, 1978].

The third reason is the differences in size of region, from which the fossil material was brought. The macrofossils from Kimmerian marine deposits contain the information about the forests, which grew near the sedimentary basin and now reflect only local vegetation of plains and lower mountain belt. The pollen and spores were brought from big distances and they allow to restore the forests of whole territory of Western Georgia from plains till upper mountain belt.

The fourth reason is the factor of time. The macrofossils are connected with separate layers, which thickness is much smaller in comparison with the thickness of whole Kimmerian stage. Naturally, they give more scanty in time imagine about vegetational cover and its dynamics than palynocomplexes, which are distributed nearly in all layers.

The comparison of Kimmerian flora with Pontian shows, that on the border of these two stretches of Pliocene nearly 120 plant species disappeared. The number of genera in families *Myricaceae, Fagaceae, Lauraceae, Aquifoliaceae, Araliaceae, Fabaceae* decreased. According to Kolakovsky [Kolakovsky, Shakryl, 1978], in spite of this in common landscape of Kimmerian Age the evergreen plants still took the significant part. This conclusion was made on the results of study of macrofossils. Judging by the palynological data, the area of subtropical plants significantly reduced after Pontian and they occupied only the relict sites, which area sometimes was widen and sometimes decreased depending on climatic conditions. At whole it is possible to speak about existence of rich warm-temperate forests with subtropical elements on plains and lower mountain belt in Kimmerian time. By Kolakovsky (1956) the composition of these forests was very variable. He distinguished three groups of plants: 1) humid monsoon climate plants; 2) hydrophilous plants, ecological similar to species of plain and riparian forests of Atlantic North America; 3) winter hardiness species, but exacting to high summer temperatures, sclerophyllous, ecological similar to components of subxerophilous forests of Mediterranean.

The warm-temperate forests occupied lower and partly the middle mountain belts. On more high levels they were changed by deciduous and coniferous formations of temperate climate. The taxonomical composition of these forests can be seen on the pollen diagram (Fig.1).

The palynological material was analyzed by means of landscape-phytocenological method. The use of the last one revealed the unstability of climate during the Kimmerian Age. The warm and humid stretches of time were changed by epochs of cool and "dry" climate. This phenomenon is the basis of subdivision of history of development of vegetation and climate on 6 stages and 14 substages (Fig.2). The warmest, close to subtropical was the climate of beginning of Lower Kimmerian (stage I) and beginning of Upper Kimmerian (stage III), to which corresponds the Duabian Flora and synchronous flora of Gulripsh. Two climatic optimums were divided by the epoch of comparable cool and less humid climate (stage II), which does not in contradiction with the idea about two waves of global warming in interval of 4.3-3.3 million years [Borzenkova, 1992]. More cold and "dry" was the stage IV, when the pine forests dominated on whole territory of Western Georgia. The warming came in stages V and VI. But they differed from I and III stages by composition of flora nearly devoted of subtropical elements.

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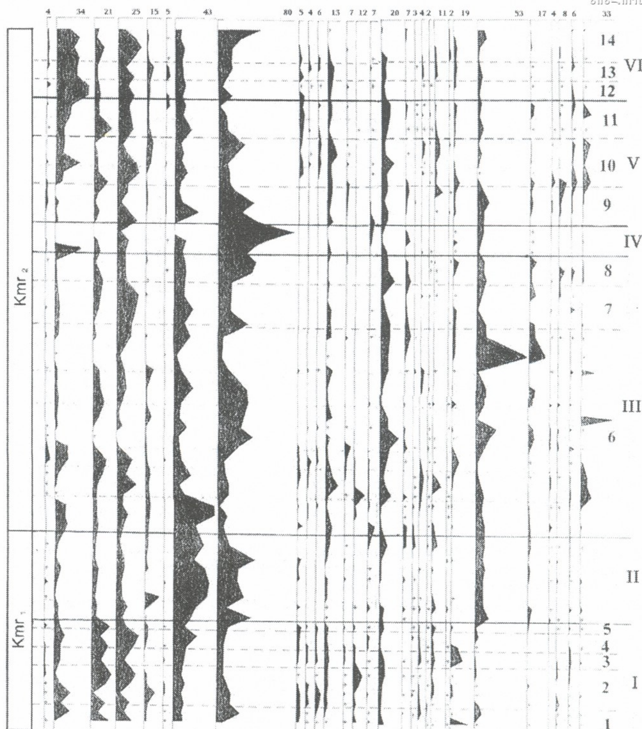

Fig. 1. Combined pollen diagram of Kimmerian deposits of Western Georgia. 4 -Betula; 34 - Picea; 21- Tsuga; 2 - Abies; 15 - Podocarpus; 5 - Dacrydium; 43 - Cedrus; 80 - Pinus; 5 - Keteleeria; 4 - Cathaya; 6 - Pseudolarix; 13 - Fagus; 7 - Carpinus; 12 - Castanopsis; 7 - Juglans; 20 - Quercus; 7 - Zelkova; 3 - Comptonia; 4 - Myrica; 2 - Hamamelidaceae; 11 - Araliaceae; 2 - Platycarya; 19 - Taxodiaceae; 53 - Carya; 17 - Pterocarya; 4 - Liquidambar; 8 - Nyssa; 6 - Ulmus; 33 - Alnus (in %).

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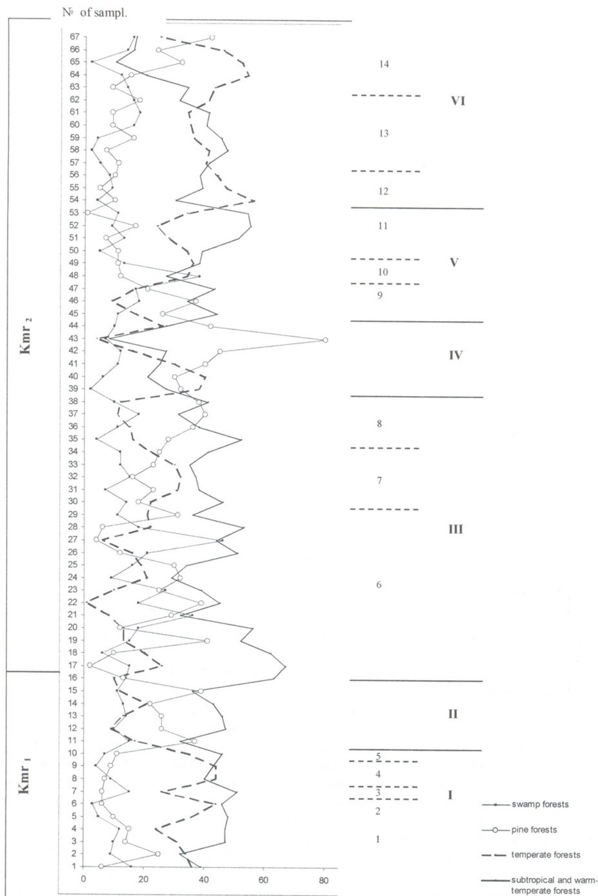

Fig.2. The correlation curves of pollen sums of the components of different ecological groups.

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The stages of development of vegetation and climate can be considered as the base of detail subdivision of Kimmerian deposits of Western Georgia on small stratigraphycal unites - palynozones (Fig.1, 2).

Conclusion

The comparison of macroflora with data of palynology revealed the big differences in composition of fossil plants. In palynocomplexes the cryptogamous plants and gymnosperms are represented by more number of forms than in the composition of macrofloras. As for the angiosperms, they are represented by nearly equal number (130-131), but the quantity of common forms determined by both methods is very small (10). The greatest similarity between macrofloras and palynofloras is traced on the level of families and genera, but the smallest - on the specific level.

In our opinion there are four main reasons, which explain the differences between macro- and palynofloras: 1) the use of different systems for determination of fossil species; 2) the different safety of fossil material; 3) the differences in size of territory from which the fossil material was came; 4) the differences in time of formation of layers with macrofossils and with pollen and spores.

The interpretation of palynological complexes via landscape-phytocenological method allows to distinguish 6 stages and 14 substages in the history of development of vegetation and climate. They reflect the epoch with different climatic conditions. Among them two climatic optimums correspond to two waves of Pliocene warming in interval 4.3-3.3 m.y.

So, only the analysis of whole paleobotanical material allows to restore the more full composition of flora and to reconstruct almost uninterrupted dynamics of vegetation and climate during one or another stretches of geological time.

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დასავლეთ საქართველოს კიმერიული ნალექების პალინოლოგიური დახასიათება

შატილოვა ი., მჭედლიშვილი ნ.

საქართველოს ეროვნული მუზეუმი, დავითაშვილის პალეობიოლოგიის ინსტიტუტი

(მიღებულია 12.03.2007)

რეზიუმე

მოცემულია კიმერიული ფლორის სრული სია. განხილულია მაკროფლორისა და პალინოლოგიური კომპლექსების სიტემატიკურ შემადგენლობებს შორის განსხვავების სავარაუდო მიზეზები. პალინოლოგიური მასალა გაანალიზებულია ლანდშაფტურ-ფიტოცენოლოგიური მეთოდის გამოყენებით. კიმერიული მცენარეულობისა და ჰავის განვითარებაში დადგენილია 6 ეტაპი და 14 ქვეეტაპი. ისინი შეიძლება განვიხილოთ როგორც საფუძველი დასავლეთ საქართველოს კიმერიული სართულის წვრილ სტრატიგრაფიულ ერთეულებად - პალინოზონებად დანაწილებისათვის.

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Zoology

SOIL NEMATODES OF SATAPLIA RESERVE

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Abstract

48 forms of soil nematodes are registered in Sataplia Reserve. Among them 37 ones are identified up to species and 11 - up to genus. They belong to 6 orders and 13 families.

Key words: soil nematodes, polyphagous, plant parasite

Introduction

Sataplia Reserve is located in West Georgia, Tskaltubo District. It occupies 354 he. Absolute height varies from 200m up to 520m [Reserves of Caucasus, 1990; Geomorphology of Georgia, 1971]. 98.3% of the territory is covered with forests. Among them 164 he is occupied with oriental hornbeam forest, 45 he - hornbeam forest, 33 he - nut wood, 28 he - chestnut forest and 27 he - beech forest.

The goal of our work is the investigation of soil nematode fauna of Sataplia Reserve, which is part of studies of Imereti fauna.

Materials and Methods

Material was collected in September, 2001 and July, 2005 in Sataplia Reserve by rout method. Soil nematodes were taken in 7 phytocenosis: oriental hornbeam, chestnut, hornbeam, beech-hornbeam (with boxwood understory), beach (from dead covering), alder and zelkova-hornbeam forests. At each site three soil samples (50 cm^3) were taken from the upper layer of soil. Nematodes were extracted by dynamic method [Metlitski, 1978], fixed in 4% formalin and temporary gliceric preparations were made according to the standard method of nematology.

Results and Discussion

List of nematodes registered on studied territory are presented in Table 1.

Table 1. Nematodes of Sataplia Reserve

Phytocenosis / Nematodes	Oriental hornbeam with Ruscus Ponticus	Chestnut wood with nut and Rododendron	Hornbeam forest with ilex and cherry-laurel understory	beech-hornbeam forest with boxwood understory	Beech forest with dead covering	Alder forest with ivy, nut, elder	Zelkova-hornbeam forest from cave
1	2	3	4	5	6	7	8
1. Menhystera villosa			+				
2. Tripylina arenicola						+	
3. Tripylina sp.		+					
4. Tripyla tenuis				+			
5. Tripyla sp.		+				+	
6. Tobrilus abberans						+	
7. Anaplectus granulosus					+		
8. Plectus annulatus		+					
9. P. parietinus	+		+				
10. Plectus sp.				+	+		
11. Nygolaimus sp.						+	
12. Mesodorylaimus bastiani	+		+		+		
13. M. mesonictius				+	+		
14. M. subtilis				+			
15. Mesodorylaimus sp.		+					+
16. Paradorylaimus longicaudatus(?)	+			+			
17. Prodorylaimus sp.				+			
18. Eudoryloimus altherri	+						
19. E. carteri		+	+	+	+	+	
20. E. centrocercus		+				+	
21. Epidorylaimus lugdunensis					+		
22. Allodorylaimus granuliferus	+						
23. Takamangai ettersbergensis	+						
24. Aporcelaimellus krigeri	+			+	+	+	+
25. A. obscuroides				+		+	
26. A. obscurus				+		+	
27. A. obtisicaudatus	+	+		+	+	+	
28. A. paraobtisicaudatus						+	
29. A. sienchorsti							+
30. A. taylori (?)							+
31. Aprcelaimellus sp.				+	+		
32. Paraxonchium striatum	+						
33. Sectonema ventcale					+		
34. Dorydorella pratensis	+				+		
35. Pungentus angulatus						+	
36. P. engadinensis						+	
37. Enchodelus hopedorus				+			
38. E. macrodorus				+			
39. Xiphinema brevicolle		+					
40. Longidorus sp.	+	+		+			
41. Clarcus papillatus		+	+		+	+	

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42. Prionchulus muscorum	+			+		+	
43. Prionchulus sp.	+						
44. Miconchus sp.				+			
45. Anatonchus tridentatus	+						
46. Tylenchorhynhus sp.	+						
47. Helicotylenchus digonicus			+				
48. Helicotylenchus sp.	+		+				
	16	10	7	17	13	15	5

Taxonomic and ecological structure of Sataplia Reserve

Registered nematodes (48 forms) belong to 6 orders (Monhysterida, Enoplida, Areolaimida, Dorylaimida, Mononchida, Tylenchida) and 13 families. Order Dorylaimida is distinguished with species diversity (23 forms). The rest orders are presented far lower. Among Dorylaimidae rich in species are genera: Qudsianematidae (6 forms), which representatives are typical edaphobionts and Aporcelaimidae (9 forms), which representatives occur in a soil, as well as in fresh water. Within them some are characterized with carnivarism, they assault Oligochaetae (*Paraxonchium striatum* and *Sectonema ventrale*). Typical predators from the order Mononchida are presented by 5 species. It should be mentioned that only 3 species of the order Tylenchida occur in our material, and species of the order Aphelenchida are not registered at all.

Sataplia Reserve nematodes belong to several ecological groups. The most diverse are omnivores and nematodes of indefinite trophic specialization to which Dorylaimidae belong. To this order also belong some phytoparasites (*Xiphinema brevicole*, *Longidorus* sp.) and predators from *Aporcelaimidae* family.

Typical soil forms are microbial phages too, which are presented by 3 orders and 10 species (Monhystera, Tripylina, Tripyla, Tobrilus, Anaplectus and Plectus genera). As we mentioned earlier, true phytoparasites from Tylenchida order are presented only by three forms (*Tylenchorhynchus* sp., *Helicotylenchus digonicus* and *Helicotylenchus* sp.).

Material obtained from various phytocenosis was rather different by nematode species composition (Table 1). As coefficient of faunal likeness Jaccard's coefficient was calculated [Chernov, 1975] (Fig.1). It was established that the likeness was very low, which we think is a result of phytocenosis differences, as well as of the fact that the material was taken once. Though, it should be noted that for some phytocenosis coefficient of faunal likeness is relatively high (e.g. beech-hornbeam and beech forest with dead covering on one hand and beech-hornbeam and alder forests on the other hand).

1	2	3	4	5	6	7
	0.08	0.15	0.13	0.20	0.10	0.05
		0.13	0.08	0.15	0.25	0.07
			0.04	0.17	0.10	0
				0.25	0.23	0.10
					0.16	0.05
						0.05

Fig. 1. Faunal likeness coefficient of phytocenosis of Sataplia Reserve

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Hitherto nematodofaunas of Tkibuli Reservoir [Japarashvili and Eliava, 1966], Imereti vineyards [Jimsheleishvili, 1999] and Racha Ridge [Kvavadze et al., 2004] are studied in Imereti. 209 forms of nematodes are registered in Upper Imereti, among them 155 forms are identified up to species.

Study of soil nematodes of Imereti is continued.

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სათაფლიის ნაკრძალის ნიადაგის ნემატოდები

ჭუჭულაშვილი ნ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 25.06.2007)

რეზიუმე

სათაფლიის ნაკრძალში რეგისტრირებულია ნიადაგის ნემატოდების 48 ფორმა, რომელთაგან სახეობამდე გარკვეულია 37, ხოლო 11 – გვარამდე. ისინი მიეკუთვნებიან 6 რიგს და 13 ოჯახს.

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Zoology

ABOUT VALIDITY OF GENUS OMODEOIA KVAVADZE, 1993 (OLIGOCHAETA, LUMBRICIDAE)

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Abstract

It was shown that earthworms genus *Omodeoia* Kvavadze, 1993 is valid taxon. Unlike other genera of the family Lumbricidae it is characterized with adjacency of the following features: setae is unpaired, clitellum is ended on 30th segment, genital setae are 3-striatal, nephridial vesicles are allantoid, basal chromosome number n=17.

Key words: *Omodeoia*, genital setae, taxon, basal chromosome number

Introduction

At the end of 20th century the new genus of earthworms (Lumbricidae) - Omodeoia [Kvavadze, 1993] was described. Not a long time ago it was invalidly considered as the synonym of the genus Dendrobaena [Csudzi and Zicsi, 2003] and only the following indication was made: "Omodeoia Kvavadze, 1993 Bull. Acad. Sci. Georgia, 148:132. syn. nov.". In our opinion such conclusion could be made only on the basis of scanning microscope researches of genital setae and analysis of obtained data. Unfortunately such studies were not carried out whereas the structure and sculpture of genital setae are valuable systematical characteristics. Their adjacency with other (ecto- and endosomatic) characteristics of the family Lumbricidae taxa - subfamilies, genera and subgenera, enables to improve and determine more precisely diagnoses [Kvavadze, 1999].

It should be also interesting to compare karyological data (chromosome number) of species of genera Omodeoia and Dendrobaena. In spite of difficulties existed while karyological studies of earthworms [Viktorov, 1993] chromosome number (somatic, basal) data are significant for decision of taxonomic and evolutionary problems of this group of invertebrates.

According to the all abovementioned we think that it is expedient to revise those characteristics, which in our opinion indicate validity of the genus Omodeoia Kvavadze, 1993.

Materials and Methods

For scanning microscopic investigations the earthworms were got from Eastern Europe till Central Asia. Genital setae of 38 species and subspecies included in the genus Dendrobaena were studied using corresponding methods [Kvavadze, 1999].

While karyological researches of species and subspecies distributed through the territory of Georgia karyotypes of species *O. byblica* and *O. arsianica* of the family Omodeoia were studied.

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The population from Gori (East Georgia) and Khelvachauri (Adjara, West Georgia) were investigated.

For chromosome preparations the methods worked out for this group of invertebrates were used [Bulatova et al., 1987].

Results and Discussion

It should be mentioned that earthworms are characterized by two types of setae: motive, i.e. locomotor and sexual, i.e. genital [Michaelsen, 1900; Stephenson, 1930]. Genital setae are collected at the front part of a body, especially at clitellum area. Papillae are developed around them. Genital setae are long, acerous in distal part and have longitudinal striae.

Stephenson [Stephenson, 1930] paid attention to the structure and sculpture of genital setae and considered that the majority of earthworm families have 4-striatal (groove) genital setae. Pop [Pop, 1952] also noticed striatal structure of genital setae. He described 4-striatal setae among the species of genus *Eiseniella*. Analogous results obtained Cernosvitov [Cernosvitov, 1937], though the number of genital seta striae among studied species was not indicated.

Following generations of scientists paid less attention to the structure and sculpture of setae except Svetlov [Svetlov, 1957], who described 3-striatal genital setae of *E. magnifica*.

According to our researches it was established that the majority of studied species of the genus Dendrobaena are provided with 4-striatal genital setae. The following 8 species and subspecies are exclusion: *Dendrobaena byblica byblica* (Rosa, 1893), *D.byblica arsianica* Kvavadze, 1985, *D. fedtschenkoi* Michaelsen, 1900, *D. hyrcanica* Kvavadze et Nikolaischvili, 1979, *D. imeretiana* Kvavadze, 1994, *D. parabyblica* Perel, 1972, *D. schelkovnikovi schelkovnikovi* Michaelsen, 1907, *D. schelkovnikovi herethica* Kvavadze 1985, in which 3-striatal genital setae are registered (Fig. 1, 2, 3 a, b). As the form, structure and sculpture of genital setae are determined genetically, those 8 species and subspecies are representatives of one definite phylogenetic branch and by the given characteristic are sharply distinguished from the rest species of genus *Dendrobaena*.

Thus, on the basis of the genital seta striatal feature and some other distinctive characteristics the abovementioned 8 species and subspecies were rightfully singled out from the genus *Dendrobaena* and united in independent genus Omodeoia Kvavadze, 1993 [Kvavadze, 1993].

We consider that species *Dendrobaena ganglbaueri* (Rosa, 1894) should be united in the genus *Omodeoia*. Its characteristic 3-striatal genital setae are clearly seen on photomaterial presented by Csudzi and Zicsi (2003) (Fig. 6.21.3; Fig. 6.21.4), whereas *D. veneta veneta*, as one of species of genus *Dendrobaena*, has 4-striatal genital setae (Fig. 6.25.3 and Fig. 6.25.4).

These data conform the consideration that the genus Dendrobaena must include only those species, which are provided with 4-striatal genital setae and the genus Omodeoia - species with 3-striatal genital setae. It should be also mentioned that in the species of genus Omodeoia the clitellum ends at 30th segment, but in the species of the genus Dendrobaena it always gets over 30th segment.

Before discussion of karyological data we should notice that the genus Omodeoia is not actually studied in this viewpoint. Only hexaploid karyotypes of species *O. byblica* and diploid karyotupes of species *O. fedtshenkoi* with basal chromosome number n=17 are studied [Omodeo, 1962; Viktorov, 1993].

By analysis of chromosomal preparations of studied species *O. arsianica* and *O. byblica* it was established that there are 17 pairs of homologous chromosomes (bivalent) in meyosis slices (plates). In mitosis metaphases 32-34 chromosomal elements are noticed.

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Obtained data confirm that species of studied populations of *O. arsianica* and *O. byblica* are diploid, where chromosome basal number is 17.

In all taxa of genus *Omodeoia* (3 species) investigated karyologically, and among them in typical species of this genus - *Omodeoia byblica*, common basal number - 17 is registered. Though species number is only one third of the total species number of this genus (9 species), according to karyological data basal number n=17 should be considered as the characteristic chromosome number for the genus Omodeoia.

It is interesting that for the genus Dendrobaena chromosome number - 18 is characteristic. It is registered in most of karyological studied taxa (14 species) and likely, evolutionary initial that chromosome number, on the basis of which development of the main species of this genus was taken place in the global lumbricofauna [Bakhtadze et al., 2005].

We should say that genera Dendrobaena and Omodeoia differ not only by genital setae and other morphological characteristics, but by chromosome basal number too. For *Dendrobaena* n=18 and for *Omodeoia* it likely equals to 17.

As a result of our studies we propose diagnosis of the given genus.

Genus *Omodeoia* Kvavadze, 1993

Diagnosis: setae are unpaired; genital setae are 3-striatal; clitellum is ended on 30th segment; 3 or 4 pairs of seminal vesicles; spermathecae ducts open above *d* setae or on *d* setae, rarely - on *c* setae; basal chromosome number n=17.

Typical species: *Allobophora (Dendrobaena) byblica* Rosa, 1893.

Other species and subspecies:

Allolobophora ganglbaueri Rosa, 1894 / = *Omodeoia ganglbaueri* Rosa, 1894 /
Allolobophora fedtschenkoi Michaelsen, 1900 / = *Omodeoia fedtschenkoi* (Michaelsen, 1900) /
Helodrilus (Dendrobaena) schelkovnikovi Michaelsen, 1907 / = *Omodeoia schelkovnikovi* (Michaelsen, 1907) /
Dendrobaena parabyblica Perel, 1972 / = *Omodeoia parabyblica* (Perel, 1972) /
Dendrobaena hyrcanica Kvavadze et Nikolaischvili, 1979 / = *Omodeoia hyrcanica* Kvavadze et Nikolaischvili, 1979 /
Dendrobaena byblica arsianica Kvavadze, 1985 / = *Omodeoia arsianica* Kvavadze, 1985 /
Dendrobaena schelkovnikovi herethica Kvavadze, 1985 / = *Omodeoia herethica* Kvavadze, 1985
Dendrobaena imeretiana Kvavadze, 1994 / = Omodeoia imeretiana Kvavadze, 1994/

Summarizing we should say that 3-striatal structure of genital setae, registered basal chromosome number n=17 of the species of genus Omodeoia and adjacency of those characteristics with other ecto- and endosomatic ones, are the basis for validity of the genus Omodeoia. At the same time, we should mention that besides species of genus Omodeoia the earthworms grouped in the genera Eisenia and Dendrodrilus are also provided with 3-strial genital setae [Kvavadze, 1996; 1997; Kvavadze, Bukhsianidze, 1996]. Genera *Eisenia*, *Omodeoia* and *Dendrodrilus* due to 3-striatal structure of genital setae were united in subfamily Eiseniinae, and at that it was noted that subfamily *Eiseniinae* Omodeo, 1956 is independent phylogenetic branch in earthworms evolution [Kvavadze, 1999].

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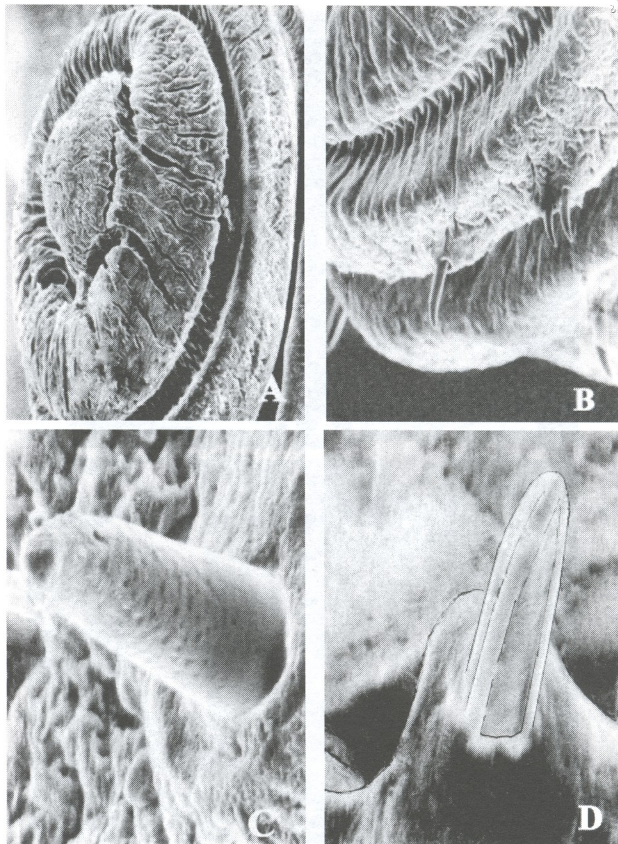

Fig. 1. *Omodeoia fedtschenkoi* (Dushambe, Tajikistan): A – prostomium × 250; B – locomotory seta 2 C (twin setae) × 500; C – locomotory seta 23 a × 2500; D – genital seta 26 b × 2500

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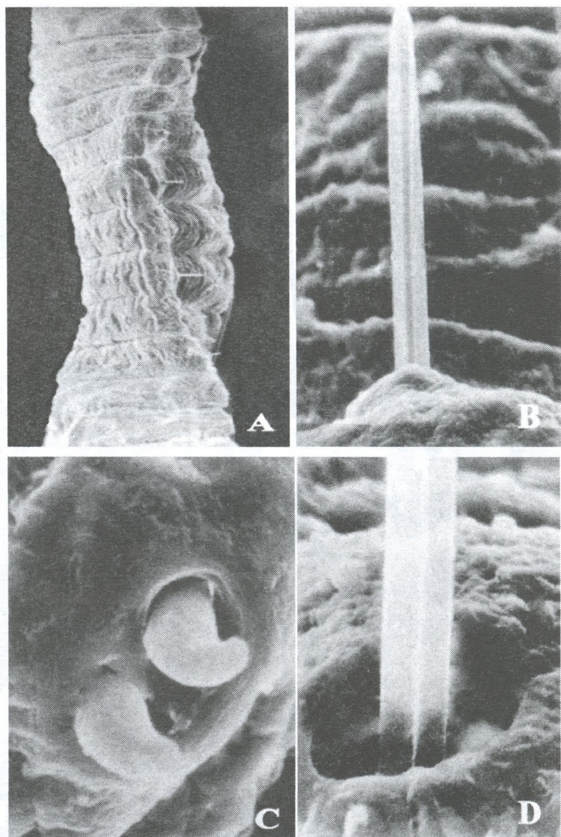

Fig. 2. *Omodeoia byblica byblica* (Gombori ridge; East Georgia): A – prostomium × 50; B – genital seta 25 b × 1000; C – locomotory seta 12 d (twin setae) × 2500; D – genital seta 28 b × 2500

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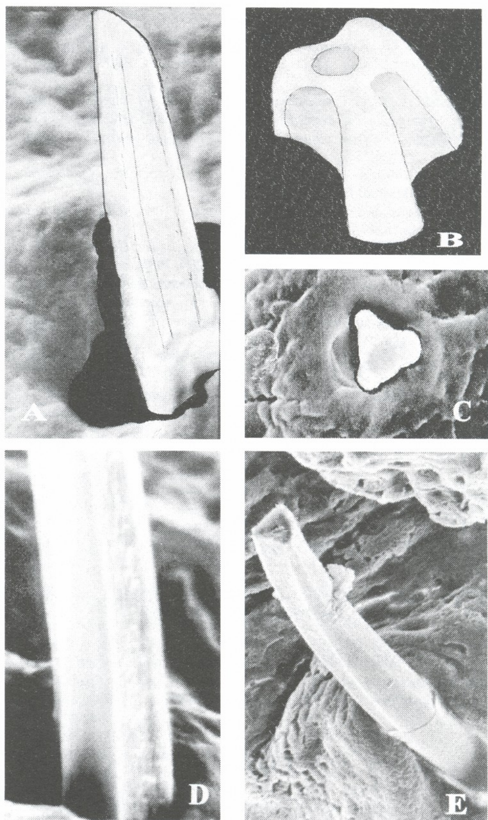

Fig. 3. The genital setae of earthworms: A – *Omodeoia bybliea byblica* (Tsalka, South Georgia), 26 a × 2500; B – *O. parabyblica* (Khosrov Reserve, Armenia), 26 a × 2500; C – *Dendrodrilus rubidus tenuis* (Voronezh, Russia), 16 a × 2500; D – *Dr. rubidus subrubicundus* (Tbilisi, Georgia), 30 b × 2500; E – *Eisenia lucens* (Parangalitsa, Bulgaria), 16 a × 2500.

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გვარის Omodeoia Kvavadze, 1993 (Oligochaeta, Lumbricidae) ვალიდურობის შესახებ

ყვავაძე ე., ბახტაძე ნ., ბახტაძე გ.

ზოოლოგიის ინსტიტუტი

(მიღებულია 11.06.2007)

რეზიუმე

ნაჩვენებია, რომ ჭიაყელების გვარი Omodeoia Kvavadze, 1993 ვალიდური ტაქსონია. ოჯახ Lumbricidae-ს სხვა გვარებისგან განსხვავებით მისთვის დამახასიათებელია შემდეგი ნიშან-თვისებების შეთანწყობა: ჯაგრები დაწყვილებული არაა, სარტყელი მთავრდება 30-ე სეგმენტზე, სასქესო ჯაგრები სამღარიანია, ნეფრიდიების ბუშტები სოსისისებრი. ბაზალური ქრომოსომული რიცხვია 17 (n).

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TWO SPECIES FROM ORDER MONONCHIDA JAIRAJPURI, 1969, FIRST FOUND IN GEORGIA

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Abstract

Soil nematodes of Gombori ridge (Eastern Georgia) have been studied. Two species from the order Mononchida - *Prionchulus auritus* and *Anatonchus ginglimodontus* are registered for the first time for the fauna of Georgia. The description, original pictures and measurements of two nematode species of the family Mononchidae are given.

Key words: Cuticle, buccal cavity, dorsal tooth, denticles, vulva.

Genus - Prionchulus (Cobb, 1916) Wu et Hoeppli, 1929

Prionchulus auritus Andrassy, 1985 [Andrassy, 1985]

Measurements:

Female: L = 2.1 mm; a = 22.7; b = 4.2; c = 13.3; v = 61.3%; c^1 = 3.3; d (anal) = 22 μm; body width = 39 μm.

Body strongly curved ventrally. Cuticle smooth. Body thickness on the middle part - 27 μm. Lips separated, labial papillae conoid, the posterior ones are ear-like, for what this species is called "auritus".

The buccal cavity large, 47-25.3 μm. Its walls moderately thick. Dorsal tooth lies over 20% of buccal cavity, two opposite arrangement of ribs of small denticles, 9-10 on each. Amphids slit-like, on the level of anterior end of mouth cavity. Oesofagus muscular, 570 μm long.

Vulva with cuticularized small knobs. Ovaries paired, outstretched and reflexed. The width of vagina equals to 2/5 of body diameter. Egg is elongated (159.6-92.4 μm) in uterus. Rectum somewhat shorter than anal body diameter. Distance between vulva and anus 4.5 times as long as tail. The latter strongly curved ventrally and finished with rounded terminus (Fig 1).

Males unknown.

Genus - Anatonchus (Cobb, 1916) de Coninck, 1939

Anatonchus ginglimodontus Mulvey, 1961 [Mulvey, 1961].

Measurements:

Female: L = 2.4 mm; a = 30.7; b = 4.6; c = 10.3; v = 63%;

Labial region distinctly separated from body contour. Buccal cavity barrel-shaped: width nearly equals to the length (44-41 μm). Three medium-sized teeth are seen quite well at the anterior wall of the buccal cavity. Wall of buccal cavity from anterior to base of teeth weakly developed. Reproductive system didelphic, ovaries reflexed. Sperm was not observed in the uterus. Tail conoid, curved ventrally. Caudal glands get into spinneret. Tail terminus rounded (Fig. 2).

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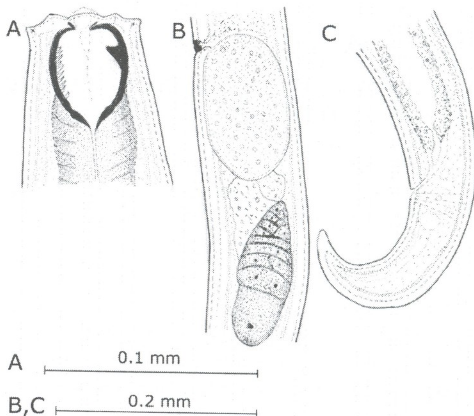

Fig. 1. *Prionchulus auritus* Andrassy, 1985
A - Had; B - Vulva region; C - Female tail.

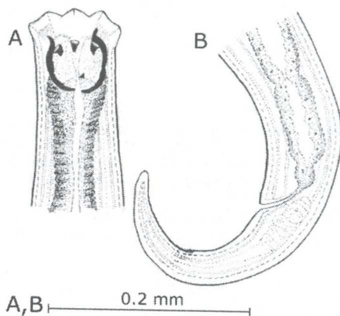

Fig 2. Anatonchus ginglimodontus Mulvey, 1961
A - Had; B - Female tail.

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საქართველოს ფაუნისათვის პირველად რეგისტრირებული ნემატოდები რიგი Mononchida-დან

ცქიტიშვილი ე.

ზოოლოგიის ინსტიტუტი

(მიღებულია 10.07.2007)

რეზიუმე

აღწერილია საქართველოს ფაუნისათვის პირველად რეგისტრირებული ნემატოდების ორი სახეობა *Prionchulus auritus* და *Anatonchus ginglimodontus.* მოცემულია მათი აღწერილობა, განაზომები და ორიგინალური სურათები.

ეროვნული ბიბლიოთეკა

ინსტრუქცია ავტორთათვის

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

წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (Introduction), მასალა და მეთოდები (Materials and Methods), შედეგები და მათი განხილვა (Results and Discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით [Lernmark, Hagglof 1981].

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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ქართული ტექსტისთვის ოპტიმალური ფონტებია AcadNusx და AcadMtavr, ინგლისური ტექსტებისთვის - Times New Roman. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც.

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

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