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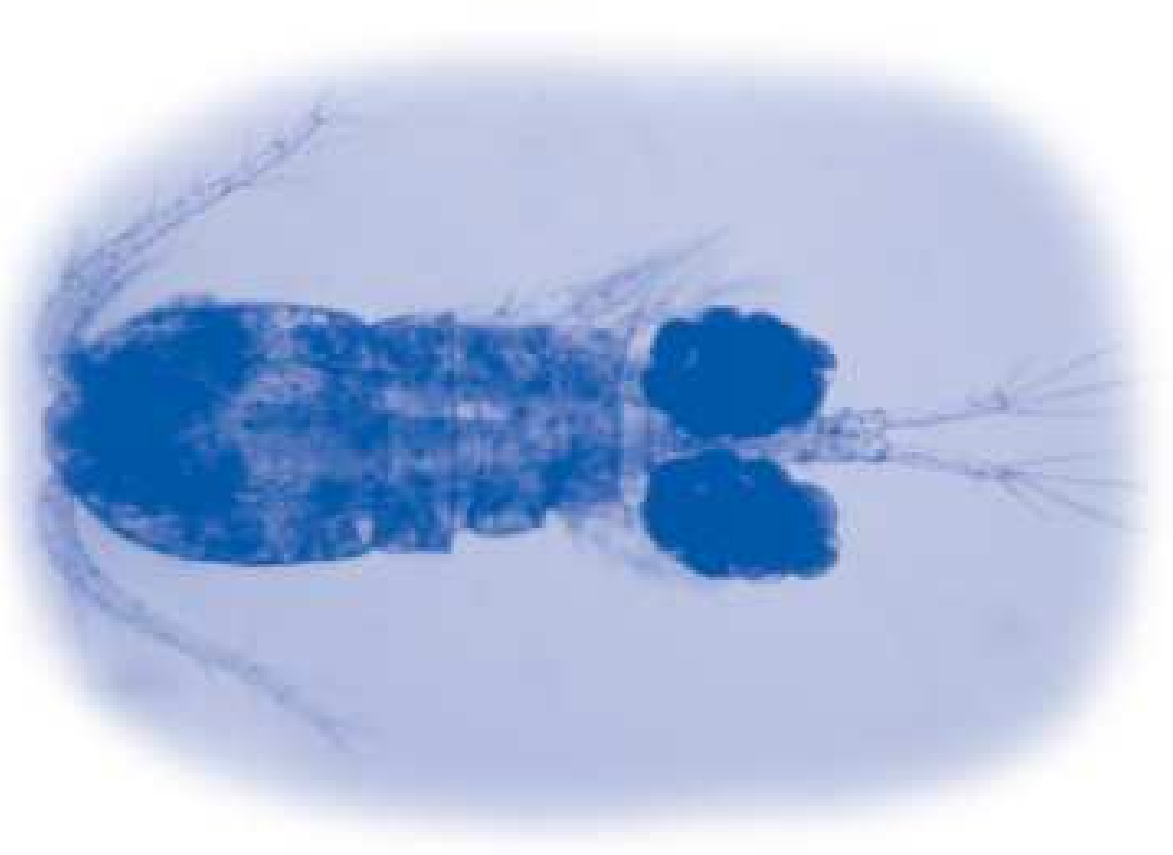

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=== SHORT COMMUNICATION ===

ZOOPLANKTONIC MICROCRUSTACEANS FROM SEVERAL GLACIAL LAKES LOCATED IN THE RETEZAT MOUNTAINS (TRANSYLVANIA, ROMANIA)

KARINA P. BATTES[1]

SUMMARY. The present paper represents a list of microcrustacean species (especially cladocerans and cyclopoid copepods) identified in 13 glacial lakes from the Retezat Mountains. The samplings took place in September 2003 and only qualitative analyses were made. Four species of cladocerans (Crustacea, Branchiopoda, Cladocera) and three of copepods (Crustacea, Copepoda, Cyclopidae) were identified in the investigated areas. Other benthic and planktonic groups were also found (on the one hand true fly larvae, Collembola, beetle larvae, ostracods and on the other hand calaniform and harpactiform copepods; rotifers).

KEYWORDS: cladocerans, copepods, cyclopoid, glacial lakes (tarns), qualitative sampling, zooplankton,

Introduction

The Retezat Mountains belong to the Western groups of the Southern-Carpathians. Here, due to the height of the mountains, the glacial relief reached the greatest expansion (Schreiber and Sorocovschi, 1993). The Retezat Mountains have the greatest number of glacial lakes in the South-Western Carpathians. There are 58 permanent and about 40 temporary tarns. The largest one is Lake Bucura (8.8 ha) while the deepest one is Lake Zănoaga (29m), followed by Lake Negru (26m) (Gîştescu 1963). Most of these lakes are located at an altitude that exceeds 1900m a.s.l (e.g. Lake Porții: 2230m).

Zooplankton of inland waters is dominated by four major groups of organisms: protists, rotifers and two groups of crustaceans that represent the topic of the present paper: cladocerans and copepods. Most cladocerans are small (0.2-3.0 mm) and feed on particles filtered from the water. Planktonic copepods consist of three major groups: the calanoids, the harpacticoids and the cyclopoids. Many cyclopoids are carnivorous on other zooplankton, some are herbivorous on a variety of unicellular and filamentous algae (Wetzel, 2001).

[1] *„Babeş-Bolyai" University, Faculty of Biology and Geology, Department of Taxonomy and Ecology, 400006, Cluj-Napoca*, E-mail: *kbattes@yahoo.com*

The first studies concerning zooplanktonic crustaceans from the Retezat Mountains were made by Daday (1879, 1883) and Szilády (1900). Subsequent information was provided by Prunescu-Arion and Toniuc (1967) and by Godeanu (1974), but these studies considered only a small number of glacial lakes.

Material and Methods

Thirteen glacial lakes were sampled in September 2003, as follows: Lake Negru, Lake Gemenele, Lake Ştirbu, Lake Agăţat, Lake Porţii, Lake Florica, Lake Viorica, Lake Ana, Lake Bucura, Lake Lia, Lake Peleguţa, Lake Peleaga and Lake Mare (Valea Rea) (Fig. 1).

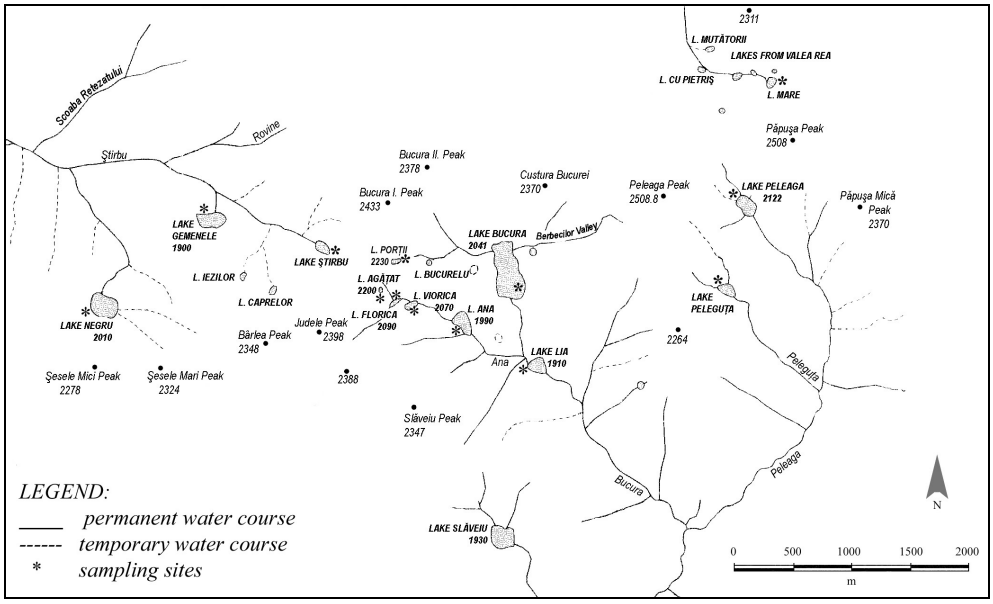

Fig. 1 The sketch map of the glacial lakes sampled in September 2003 (marked with asterisk) (redrawn from Bartos et al., 2003)

Zooplanktonic microcrustaceans were sampled using a 50 µ mesh size plankton net. The samples were preserved with sucrose solution according to Haney & Hall method (Haney and Hall, 1973), and in 4% formaldehyde. Identifications were made to the species level in case of cladocerans (Negrea, 1983; Dumont and Negrea, 2002) and cyclopoid copepods (Damian-Georgescu, 1963; Dussard and Defaye, 2001). Rotifers, ostracods, harpacticoid and calanoid copepods, collembola, beetle larvae and true fly larvae were also found in the samples collected from the thirteen glacial lakes.

Results and Discussion

Four species of cladocerans and three species of cyclopoid copepods were identified in the tarns considered for the present study. Table I presents the taxonomic groups collected from the glacial lakes sampled in September 2003.

Table 1.

List of taxonomic groups identified in thirteen glacial lakes from the Retezat Mountains sampled in September 2003

	Taxonomic group	Sampled lakes												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Cladocera	*Daphnia obtusa* Kurz							♀					♀	♀
	Daphnia longispina O.F. Müller	♀		♀♂	♀				♀			♀		
	Chydorus sphaericus (O.F. Müller)	♀	♀	♀	♀	⚥	♀	♀	♀	♀	♀	♀	♀	♀
	Alona affinis (Leydig)	♀	♀				♀	♀	♀	⚥	♀		♀	
Copepoda Cyclopidae	*Eucyclops serrulatus serrulatus* (Fisher)	♀		♀	♀		♀			♀	♀			
	Acanthocyclops vernalis vernalis (Fisher)	♀		♀						♂	♀	♀♂	♂	
	Acanthocyclops crassicaudis crassicaudis (Sars)							♂						
	Copepodites													
	Nauplii													
Others	Rotifera													
	Ostracoda													
	Copepoda Calaniformes													
	Copepoda Harpacticoida													
	Collembola													
	Coleoptera (larvae)													
	Diptera (larvae)													

Footnotes:
1 - Lake Negru; 2 - Lake Gemenele; 3 - Lake Ştirbu; 4 - Lake Agăţat; 5 - Lake Porţii; 6 - Lake Florica; 7 - Lake Viorica; 8 - Lake Ana; 9 - Lake Bucura; 10 - Lake Lia; 11 - Lake Peleguţa; 12 - Lake Peleaga; 13 - Lake Mare (Valea Rea)
⚥ - parthenogenetic and gamogenetic females in cladocerans; ♀,♂- females and males in copepods;
The grey areas indicate the presence of taxonomic groups.

The following cladoceran species were identified: *Daphnia obtusa* Kurz and *Daphnia longispina* O.F. Müller, belonging to Family Daphniidae, and *Chydorus sphaericus* (O.F. Müller) and *Alona affinis* (Leydig) from Family Chydoridae. Cyclopoid copepods included *Eucyclops serrulatus serrulatus* (Fisher) (Subfamily Eucyclopinae) together with *Acanthocyclops vernalis vernalis* (Fisher) and *Acanthocyclops crassicaudis crassicaudis* (Sars) (Subfamily Cyclopinae).

In case of cladocerans, both parthenogenetic and gamogenetic females were found. Males were identified only in case of *Daphnia longispina* collected from Lake Ştirbu. Ephippial eggs were present in most lakes (lakes 1, 2, 3, 6, 7, 8, 9 and 10 from table I).

Young stages of copepods, naupliar larvae and copepodites, were collected from every lake. Adult individuals were represented by females, both ovigerous and non-ovigerous, and males (see table I).

In general, the cladoceran species presented above are characteristic for glacial lakes; only *Chydorus sphaericus* is cosmopolite. Cyclopoid copepods collected from the sampled tarns can withstand large variations of ecological factors, except for *Acanthocyclops crassicaudis crassicaudis*, which is a stenoterme species, preferring warmer waters.

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PHYTOSOCIOLOGICAL STUDY OF THE SWAMP FROM TÂRSA BĂLCEŞTILOR (VĂLENILOR), CLUJ COUNTY

GHEORGHE GROZA[1], **PAVEL-DAN TURTUREANU**[1]

SUMMARY. This study analyses the character of vegetation on an area of about 10 ha, situated between Beliş and Bălceşti village – Cluj district. Ecologically, it occupies an intermediary position between a meso-eutroph swamp to an peat bog. In view of phytosociologic characterisation, in august 2007, a number of 39 relevees were realized, after the classical method Braun-Blanquet, used in the majority of phytosociologic studies. Later, using statistical software, based on the synthetic table of these relevees, a graphical representation of type DCA (Detrended Correspondence Analysis) was obtained. The vegetation analysis based on the phytosociologic relevees, led us to classifying vegetation in 8 associations, comprised in 3 classes of vegetation.

KEYWORDS: vegetation, mountain level, meso-eutroph swamp, oligotroph swamp

Introduction

The studied surface is situated between Beliş and Bălceşti village, in Cluj district. The toponyme used to appoint it is "Târsa Bălceştilor (Vălenilor)". The access is easy, from the communal road that links the two localities, before the entrance in Bălceşti, on the right side of the road.

The perimeter is a surface of about 10 ha, at the spring of a small river that flows 2-3 km up to the confluence with the small river Călățele, with the main flowing way N-NE.

The general slope of the considered surface is very small (approx. 6%), and the exposition is N-NV. The medium elevation of 1135 m makes the surface to be integrated in the lower level of spruce fir, from the point of view of altitudinal zone. The lower half of the considered valley is covered by forest and toward the spring there are wide surfaces of grazing field and communal hayfields, observing the "attempt" of expansion and recolonizing of the field by the forest, by spruce firs disseminated in groups. The majority of liftings were on the right side of the upper valley, close to the road. This part has a hydromorphic soil formed by the flows from the superficial springs. The accumulation of water corroborated with the substrate's acidity formed by schists, led to formation of a moss layer composed of species of *Sphagnum.*

[1] *University of Agricultural Sciences and Veterinary Medicine, 3 Mănăştur Street, 400372/Cluj-Napoca, Romania, E-mail: turtureanudan@ gmail.com*

Fig. 1. General view of the studied perimeter.

The physiognomy of vegetation has a mosaical character, with small groups or isolated individuals of spruce firs and bushes of *Salix cinerea,* based on three formations enlightened by two species of rush *Carex echinata* and *C. rostrata* and by one specific species of peat bogs, *Eriophorum vaginatum,* all three being constantly followed in the moss layer by species of the kind *Sphagnum* (Fig. 1).

The surrounding area of the swamp is mostly occupied by acidophile grazing fields. (with *Nardus stricta, Calluna vulgaris, Campanula abietina* etc.), developed on the place of old cleared forests. The link betweeen these grazing fields that are now influenced by overgrazing and the studied swamping is realized by a buffer zone with intermediary character.

Material and Methods

The utilised method in the study of vegetation was a classical one, using the relevee technique, followed by a statistical multivaried analysis based on the data extracted from the synthesis of phytosociologic tables (relevees). Evaluation of the abundance-dominance index (AD) in the scale Braun-Blanquet confers values from 1 to 5, with r being noted the species with a covering between 0.01-0.1% and with + the ones between 0.1-1% from the total surface of the relevee.

According to the size of relevees, these had an area of 25 m^2. The relevees were realized in the period 15-20 august 2007.

In view of realizing the multivaried analysis, the specific notes of the scale Bran-Blanquet from the relevee tables were reduced to a scale from 1 to 6, and so, the classes r and + from the noting system Blanquet were substituted with 1 in view of reducing the dispersions appeared in the area of the flow charts.

The ulterior analysis of the synthesis table formed from the total of relevees, transformed according to the new scale, was realized using the functions of the kit PAST 1.72 (Palaeontological Statistics). As a result of some more attempts to obtain an ordinogramme that would offer a suggestive explanation of the ecological conditions of the studied area, it was opted for the presentation of a – **DCA** – Detrended correspondence analysis (Hill et. Gauch, 1980).

Results and Discussion

Based on the relevees realized on the field, we identified 8 associations comprised in the following cenotaxonomic summary:

The cenotaxonomic summary of vegetable associations

SCHEUCHZERIO – CARICETEA *Fuscae* Tx. 1937
CARICETALIA FUSCAE Kick 1926
Caricion fuscae Koch 1926 em. Klika 1934
1. ***Sphagno-Caricetum rostratae*** Steffen 1931
2. ***Carici echinatae-Sphagnetum*** (Balázs 1942) Soo 1955
OXYCOCCO – SPHAGNETEA Br.-Bl. et Tx. ex Westoff et al. 1946
SPHAGNETALIA MAGELLANICI (pawl. 1928) Moore 1968
Sphagnion magellanici Kastner et Flosner 1933
3. ***Eriophoro vaginati – Sphagnetum recurvi*** Hueck 1925
MOLINIO-ARRHENATHERETEA Tx. 1937
MOLINIETALIA Koch 1926
Molinion caerulae Koch 1926
4. ***Junco- Molinietum*** Preising 1951
5. ***Holcetum lanati*** Issler 1936 emend. Pass. 1964
POTENTILLO-POLYGONETALIA R.Tx.1947
Potentillion anserinae R.Tx. 1937
Juncenenion effusi Westhoff et van Leeuwen ex Hejny et al. 1979
6. ***Juncetum effusi*** Soo (1931) 1949
Calthion R. Tx. 1937
7. ***Scirpetum sylvatici*** Ralski 1931, Maloch 1935 em. Schwich 1944
DESCHAMPSIETALIA CAESPITOSAE Horvatic 1956
Deschampsion caespitosae Horvatic 1930
8. ***Deschampsietum caespitosae*** Horvatic 1930

The characterization of associations:

1**. *Sphagno-Caricetum rostratae*** Steffen 1931(Tab. 1, rel.1-6)

Carex rostrata is a species with a large amplitude according to the hydric regime, populating drained but also flooded places (Lupşa, 1971). This association is also met in lower zones having a mesotroph character. Here, the enlightened coenosis by this species that is usually in a relation of codominance with a layer of moss formed by *Sphagnum*, evolves into the formations of oligotrophic–type

(*Eriophoro vaginati – Sphagnetum recurvi).* In this way, the extension of *Carex rostrata* groups precedes the installation of *Sphagnum* communities (Boşcaiu, 1971).

A well defined fact in this case was the more pronounced humidity of the soil and eventually some stagnations of water after a drought time, in areas where this species is more abundant. The coenosis of this vegetable group are met near the water but more in the high parts of the right side of the mountain.

The physiognomy of the grassy layer is marked on the entire studied area by the dominant species, with larger leaves and the bluish-green tint, compared with the second predominant element in the area, *Carex echinata.*

An interesting fact in the structure of these phytocoenosis was the permanent presence of a assemblege of species belonging to the class *Molinio-Arrhenatheretea.*

2. ***Carici echinatae-Sphagnetum*** (Balázs 1942) Soo 1955 (Tab. 1, rel. 7-12)

The phytocoenosis of this association are spread predominantly in areas with high and very high acidity, where the peat layer isn't very thick. The pillows of *Sphagnum* are in an invasive process, and once with the peat progression this association is decreasing (Lupsa, 1971).

Specific transgressive species to the *Molinietalia* order are diffusing in association with a considerable number of species in *Calthion* order but with a lower consistency, decreasing with the dominance of *Sphagnum* and increasing of oligotrophicity. Interestingly, in these relevees *Nardus stricta* is not present, but appears in the old centers of swamping, indicated by the presence of *Eriphorum vaginatum*. It is possible that this is caused by their closeness with the surrounding grazing fields.

3. ***Eriophoro vaginati – Sphagnetum recurvi*** Hueck 1925; (Tab. 2, rel.17-25)

This association is typically oligotroph, consisted of installed phytocoenosis on acide soil and with a low trophicity. It is met in isolated groups, with a small affinity to coenosis enlightened by *Carex. rostrata*, a fact sometimes distinguished by multivariate analyses. In the field, despite all efforts this scanning could not be made visually.

Table 1

Phytocenoses of associations *Sphagno-Caricetum rostratae* Steffen 1931 (rel. 1-6) and *Carici echinatae-Sphagnetum* (Balázs 1942) Soo 1955 (rel. 7-12)

Relevee	1	2	3	4	5	6	7	8	9	10	11	12
Altitude (m)	1130	1130	1132	1135	1130	1125	1125	1130	1130	1130	1120	1120
Aspect	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV
Slope (degrees)	5	5	5	6	6	5	5	5	4	5	5	5
Cover %	100	100	100	100	100	100	100	100	100	100	100	100
Sphagnum sp.	3	3	4	3	4	4	3	4	4	3	3	4

Table 1 (continued)

Carex echinata	.	+	.	+	.	.	4	3	3	3	3	4
Carex rostrata	4	4	4	4	3	4	.	1	+	r	.	+
Agrostis canina	r	.	.	.	+	+	.	+	r	.	+	+
Anthoxanthum odoratum	.	.	.	.	.	.	.	r	+	.	.	.
Briza media	.	.	.	.	.	.	.	.	.	.	.	+
Caltha palustris	+	.	.	.	.	+	r	+	.	r	.	+
Chaerophyllum hirsutum	+	.	.	.	.	.	.	.	r	.	.	.
Cirsium rivulare	r	.	.	.	.	.	r	.	+	.	.	+
Crepis paludosa	+	1	.	1	.	.	+	+	+	+	.	+
Cynosurus cristatus	.	.	.	1	.	.	.	.	.	.	.	.
Epilobium palustre	+	.	.	.	.	.	.	+	+	r	.	.
Equisetum pratense	+	+	1	+	+	+	+	.	+	r	+	.
Eriophorum latifolium	.	.	.	.	.	.	.	.	.	.	.	1
Eriophorum vaginatum	.	.	.	.	.	+	.	.	.	.	.	.
Festuca rubra	.	+	.	+	.	.	.	+	r	+	.	.
Fillipendula ulmaria	.	.	.	.	.	.	.	+	.	.	.	.
Galium uliginosum	.	.	.	.	.	.	.	+	.	.	.	.
Juncus effusus	r	.	.	.	r	r	r	.	.	.	r	.
Knautia arvensis	.	.	.	.	.	.	.	+	.	.	.	.
Lychnis flos-cuculi	.	.	.	.	.	.	.	.	r	.	.	.
Lysimachia vulgaris	.	.	.	.	.	.	.	.	.	.	.	+
Myosotis palustris	.	.	.	.	.	.	.	.	.	r	.	.
Oenanthe aquatica	r	.	.	.	.	.	.	r	.	.	.	.
Orchis maculata	.	.	.	.	.	.	r	.	.	.	.	.
Potentilla erecta	.	+	.	+	r	.	r	r	.	.	r	+
Ranunculus repens	r	.	.	.	.	.	.	.	.	.	.	.
Salix cinerea	r	r	.	r	.	.	r	.	r	+	.	.
Sorbus aucuparia	.	r	.	r	r	.	.	.	.	.	r	.
Succisa pratensis	.	.	.	.	.	.	.	.	.	.	.	+
Vaccinium myrtillus	.	.	+	.	+	.	.	r	.	r	+	.
Vaccinium vitis-idaea	.	+	+	+	+	.	r	r	.	r	+	r

This is because of the ecological parameters that are pretty homogenous, especially respecting the trophicity, all groups being close from this point of view. The direct separation could not be made in an evident way.

4. ***Junco- Molinietum*** Preising 1951; (Tab. 2, rel.13-16)

Table 2

Phytocenoses of associations *Eriophoro vaginati – Sphagnetum recurvi* Hueck 1925 (rel. 17-25) and *Junco- Molinietum Preising* 1951 (rel. 13-16)

Relevee	17	18	19	20	21	22	23	24	25	13	14	15	16
Altitude(m)	1120	1120	1125	1125	1130	1130	1130	1125	1125	1130	1130	1130	1130
Aspect	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV
Slope(degrees)	4	4	4	5	5	4	6	6	6	5	5	5	5
Cover %	100	100	100	100	100	100	100	100	100	100	100	100	100
Sphagnum sp.	3	3	3	3	4	4	4	3	4	2	3	3	2
Carex echinata	.	1	+	+	2	2	1	+	+	1	.	+	1
Carex eostrata	.	.	.	.	.	.	.	r	.	1	+	1	.
Agrostis canina	+	+	.	.	.	+	1	1	+	+	.	1	+
Anthoxanthum odoratum	+	+	.	.	.	+	+	.	.	.	.	.	+
Crepis paludosa	+	.	.	.	.	1	.	.	+	+	+	.	.
Deschampsia flexuosa	1	+	1	+	+	+	.	+	.	.	+	.	+
Epilobium palustre	.	.	.	.	.	+	.	.	+	r	.	.	.
Equisetum pratense	1	.	+	.	+	+	.	.	1	+	+	.	.
Eriophorum latifolium	.	.	.	.	.	.	1	1	1	.	.	1	.
Eriophorum vaginatum	3	3	3	3	3	3	3	2	2	.	r	.	.
Festuca rubra	.	+	.	.	.	+	+	+	+	r	r	+	.
Juncus effusus	+	+	+	+	r	1	+	+	+	1	2	+	1
Knautia arvensis	.	.	.	.	.	.	.	.	.	+	.	.	.
Lysimachia vulgaris	.	.	.	.	.	.	.	.	.	.	.	+	.
Molinia caerulea	.	.	.	.	.	.	+	r	.	3	3	3	3
Nardus stricta	.	+	.	.	.	.	+	r	.	.	.	+	+
Orchis maculata	.	.	.	.	.	.	.	.	.	r	.	.	.
Picea abies	.	.	.	.	+	.	.	.	.	.	.	.	.
Potentilla erecta	+	+	.	r	.	+	+	+	.	+	+	+	+
Succisa pratensis	.	.	.	.	.	.	.	.	.	.	+	.	.
Vaccinium myrtillus	+	+	+	+	+	.	.	.	.	.	.	.	.
Vaccinium vitis-idaea	.	.	.	.	+	r	.	.	.	.	+	.	+
Veratrum album	.	.	.	.	.	.	.	.	.	r	.	.	.

The small enlightened groups of *Molinia caerulea* are very weakly represented. The association was identified only isolated, dispersed on the field in a pretty chaotic way, having in its structure species as *Juncus effusus* (sometimes dominantly), *Potentilla erecta, Veratrum album* etc.

From the ordinations analysis, resulted that relative to the other vegetable groups, the *Molinia caerulea* association was placed in an intermediary position.

5. ***Holcetum lanati*** Issler 1936 emend. Pass. 1964 (Tab. 3, rel. 38-39)

It was met sporadically in the close neighborhood of the small river, in groups of just a few square meters. The very weak presence is explained by the species' preferences for the low-moderate acid substrates. It forms stable coenosis at low altitudes, in wet watersides

6. ***Juncetum effusi*** Soo (1931) 1949 (Tab. 3, rel. 30-35)

The presence of these communities in the considered area, becomes evident near the river and in bordering parts of the swamp, being distinguished as having affinities with the groups dominated by *Deschampsia caespitosa* and *Holcus lanatus*.

Compact groups of this species colonize the pretty beaten field of grazing fields in the vicinity, especially around the peat bog, this phenomenon being very frequent in the region. This abundant developing of shave grass is probably stimulated by overgrazing. The species doesn't manifest any preference for another factor than humidity.

7. ***Scirpetum sylvatici*** Ralski 1931 (Tab. 3, rel. 36-37)

It was identified only in some points, in the middle side of the small river course, at the beginning of the forest, in the most northern point of the studied area. It forms short strips, in places diffusely illuminated.

8. ***Deschampsietum caespitosae*** Horvatic 193. (Tab. 3 , rel.26-29)

It is localized in small groups representative only near the water, on the left side of the mountain. The swamping is no longer felt as on the right side of the mountain, and the transition toward mesophilous meadows near the swamp is made in a more sudden way. Coenosis enlightened by tufted hair grass (*Deschampsia caespitosa*) are at the contact between riparian communities groups, represented on narrow insignificant strips, and mesophilous meadows. A large number of components come from these associations that are interdependent with *Deschampsia*.

On this side of the mountain, along the small river, on a muff of a few meters, exists a gradual alternation between the groups of *D. caespitosa* and of *Juncus effusus*. As the ecological conditions are somehow identical, this way of positioning is most probably due to the competition between the two species.

Table 3

Phytocenoses of associations *Deschampsietum caespitosae* Horvatic 1930 (rel.26-29), *Juncetum effusi* Soo (1931) 1949 (rel.30-35), *Scirpetum sylvatici* Ralski 1931 (rel.36-37), *Holcetum lanati* Issler 1936 emend. Pass. 1964 (rel.38-39)

0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Relevee	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Altitude(m)	1130	1130	1130	1130	1130	1130	1130	1125	1125	1125	1130	1130	1130	1130
Aspect	N	N	N	NE	NV	NV	NV	NV	NV	NV	NV	NV	NV	NV
Slope(degrees)	2	2	2	2	4	5	5	5	5	6	6	6	6	6
Cover %	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Sphagnum sp.	.	.	.	.	2	3	3	2	2	2	3	3	.	.
Carexc echinata	2	2	2	1	.	.	.	+	+	.	+	+	1	1
Carex rostrata	.	.	.	.	.	.	+	.	.	.	+	1	.	+
Agrostis canina	1	+	1	+	1	+	+	.	.	.	+	r	r	+
Agrostis tenuis	.	.	.	.	.	.	.	+	+	+	.	.	.	+
Anemone nemorosa	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Anthoxanthum odoratum	1	+	1	+	+	.	1	+	+	.	.	.	r	+
Briza media	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Caltha palustris	.	.	.	.	.	.	.	.	.	.	+	.	r	.
Carex leporina	+	r	.	+	.	.	.	.	.	.	r	r	+	.
Carex fusca	.	.	r	.	.	.	.	.	.	.	.	.	.	.
Chaerophyllum hirsutum	.	.	.	.	.	.	.	.	.	.	.	.	r	.
Cirsium rivulare	.	.	.	.	.	.	.	.	.	.	r	r	.	.
Crepis paludosa	+	r	.	.	.	.	.	.	.	.	+	r	r	+
Cynosurus cristatus	.	.	.	.	.	.	r	.	.	.	.	.	r	+
Deschampsia caespitosa	3	3	2	2	.	.	+	.	.	.	.	r	.	.
Deschampsia flexuosa	.	.	.	r	1	1	+	2	1	+	.	.	.	.
Driopteris carthusiana	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Epilobium palustre	.	r	.	.	.	.	+	.	.	.	+	r	.	+
Equisetum pratense	r	r	+	.	.	r	.	.	.	+	.	r	.	.
Eriophorum latifolium	.	.	.	.	.	+	.	.	.	.	.	.	.	.
Eriophorum vaginatum	.	.	.	.	r	.	.	.	.	.	.	.	.	.

Table 3 (continued)

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Festuca rubra	.	.	+	.	+	+	.	.	.	.	.	.	.	.
Fillipendula ulmaria	.	.	.	.	.	.	.	.	.	.	+	+	.	r
Galium uliginosum	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Galium palustre	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gentiana cruciata	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Holcus lanatus	.	.	.	.	.	.	.	.	.	.	r	.	3	3
Homogyne alpina	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hypericum maculatum	.	.	.	.	.	.	.	.	.	.	r	+	r	.
Juncus articulatus	.	.	.	.	.	.	.	.	.	.	.	r	.	.
Juncus subnodulosus	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Juncus effusus	+	1	+	1	3	3	4	3	3	4	1	+	1	+
Knautia arvensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Luzula luzuloides	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lychnis flos-cuculi	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lysimachia vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Molinia caerulea	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Myosotis palustris	.	.	.	.	.	.	.	.	.	.	r	+	.	.
Nardus stricta	+	+	+	+	r	.	.	+	+	.	.	.	+	r
Oenanthe aquatica	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Orchis maculata	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Picea abies	.	.	.	.	r	.	.	.	.	.	.	.	.	.
Potentilla erecta	+	r	+	+	+	.	r	+	+	+	.	r	+	r
Prunella vulgaris	.	.	.	.	.	.	.	.	.	.	.	.	r	.
Ranunculus acris	r	+	.	.	.	.	+	.	.	.	.	.	+	.

Table 3 (continued)

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ranunculus repens	.	.	.	.	.	.	.	.	.	.	.	r	+	r
Salix cinerea	.	.	r	.	.	.	.	.	.	.	+	r	.	r
Salix purpurea	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Scirpus sylaticus	.	.	.	.	.	.	.	.	.	.	3	3	.	.
Sorbus aucuparia	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Stellaria graminea	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Succisa pratensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium alpestre	.	.	.	.	.	.	.	.	.	.	+	r	r	.
Vaccinium myrtillus	.	.	.	.	+	.	.	+	r	.	.	.	.	.
Vaccinium vitis-idaea	.	.	.	r	+	+	r	+	+	+	.	.	.	.
Veratrum album	.	.	.	.	.	.	.	.	.	.	.	.	.	.

The graphical display reveals the main affinities between samples of different cenotaxonomic groups, and especially the relation between these and certain ecological gradients, normally associated with the two axes of the ordination diagram (Fig. 2). As the ecological conditions were pretty uniform and the studied area is small, these gradients did not have an obvious amplitude. The only visible differences, but in a small measure, were caused by the degree of humidity and oligotrophism. This way, the vertical axis correlated with the change in the degree of humidity, observed most times on the field too, culminating with the small water pounds, on which were found mostly the communities of *Carex rostrata*, and at the other end, on more inclined parts, phytocoenosis of *Deschampsia caespitosa*.

The multivariate analysis

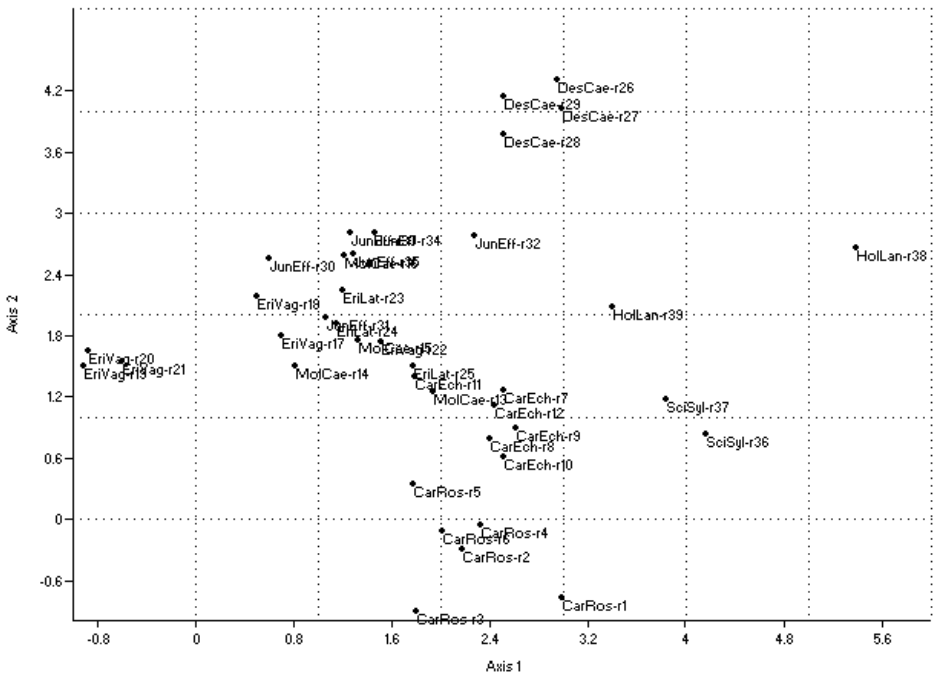

Fig. 2. The multivariate analysis of type DCA – Detrended correspondence analysis, based on quantitative data of relevees from the phytocoenoses present in the studied area. The relevees were symbolized this way: CarRost-r/nr for Rel.1-6, CarEch-r/nr for Rel.7-12, EriVag+EriLat-r/nr for Rel.17-25, MolCae-r/nr for Rel.13-16, DesCae-r/nr for Rel.26-29, JunEff-r/nr for Rel.3.-35, SciSyl-r/nr for Rel.36-37, HolLan-r/nr for Rel.38-39.

The second axis of the system, horizontal (Axis 1), correlated with the degree of oligotrophism of the habitats. The associations enlightened by the specific element of the oligotroph swamps (*Eriophorum vaginatum*) are situated at the other end of the dominants *Holcus lanatus* and *Scirpus sylvaticus*, indicators of some swampings yet unaffected by the peat phenomenon.

The relevees dominated by *Molinia caerulea* and *Juncus effusus* don't occupy well spatial defined positions, which is explained by the ecological intermediary position of the studied area, between the swamping eu-mesotroph lawns and oligotroph peat bogs. The two species, but especially their companions, were affirmed as having higher ecological amplitudes.

Conclusions

The studied area, situated in the place called "Târsa Bălceştilor (Vălenilor)" covers an ensemble of eight plant associations, of which three (*Sphagno-Caricetum rostratae, Carici echinatae-Sphagnetum, Eriophoro vaginati–Sphagnetum recurvi)* occupy large areas and confer the general physionomy of the field and other five, that were settled because of the presence on small, scattered areas, with different ecological conditions: *Deschampsietum caespitosae, Juncetum effusi*, *Scirpetum sylvatici*, *Holcetum lanati, Junco-Molinietum*.

Ecologically, the flora and vegetation analysis revealed different stages of transition from eutroph swamps to the oligotroph ones peatbogs with *Eriophorum vaginatum*). The multivariate analysis shows the mosaical character of vegetation, caused by humidity and chemical reaction factors of the substrate.

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THE ACTIVITY OF THE ANTIOXIDATIVE ENZYMES IN THE *MOUGEOTIA* ALGAL SUSPENSIONS, STRAIN AICB 560, UNDER LIGHT STRESS AND LOW TEMPERATURES

VICTOR BERCEA[1], BOGDAN DRUGĂ[1, 2]
NICOLAE DRAGOŞ[1, 2]

SUMMARY. The activity of antioxidative enzymes was investigated under light stress, low temperatures and in the presence of certain photosynthetic inhibitors, during the state transitions of the exponentially *Mougeotia* cultures. The activity of SOD and GR has been enhanced, excluding the anaerobiosis conditions induced by FCCP, which have lead to the decrease of oxygen production - the main source of the reactive oxygen species. Blocking the PS II by DCMU, the cyclic electrons flow around the PS I operates at highest rate. In the conditions of intense light, low temperature and anaerobiosis, the activity of the ascorbate peroxidase has been intensified. The activity of monodehydroascorbate reductase has decreased, excepting the state of anaerobiosis. Moreover, the activity of dehydroascorbate reductase and NADPH dehydrogenase has significantly decreased both under intense light and low temperatures, and also in anaerobiosis or in the state transition of the photosystems. Under light stress and low temperature, the lipids peroxidation has been enhanced, probably due to the oxidative damages. When the photosynthetic inhibitor DCMU has been added during the *state 2*, the lipids peroxidation activity has been enhanced two times. The maintenance of the electrons flow from the thylakoids is essential for the chloroplasts protection against the photooxidative stress, this being a process in which the cyclic electrons flow and the water-water cycle also contribute.

KEYWORDS: ascorbate peroxidase; monodehydroascorbate reductase; dehydroascorbate reductase; superoxyde dismutase; glutathione reductase; NAD(P)H-dehydrogenase; lipid peroxidation

Introduction

The low temperature induces the decrease in the CO_2 assimilation together with the inhibition of photosynthesis, leading to the enhancement of the excess energy dissipation in the PS II antenna, and to photodamages in the PS II reaction center (Fryer *et al*., 1998), and also to the decrease of the Calvin cycle enzymes (Kingston-Smith, 1997).

[1] *Institute of Biological Research, 48 Republicii Street, 400015/Cluj-Napoca, Romania*
E-mail: bercea_victor@yahoo.com
[2] *Faculty of Biology and Geology, Babeş-Bolyai Univ., 5-7 Clinicilor Street, Cluj-Napoca, Romania*

The unfavorable environmental conditions make the photosynthetic electrons flow towards O_2 to enhance, causing the increase of the superoxide, H_2O_2 and hydroxyl production, which leads to the limitation of the $NADP^+$ concentration in the acceptance of electrons from the PS I (Asada, 1999). In anaerobiosis, the ATP synthesis occurs without to influence the NADP/NADPH ratio due to the operating of the electrons cyclic flow around the PS I (Forti *et al*., 2003).

The O_2 reactive species are quickly removed inside of chloroplasts by a group of enzymes consisting in SOD, GR, DHAR, MDHAR and APX (the chloroplasts lack catalase) (Asada, 1999). The increase in concentration of these enzymes may limit the photodamages (Hodges *et al*., 1997). In normal conditions, the majority of the reductants that are generated by the electrons transport are consumed during the CO_2 assimilation, while other pathways such as the nitrogen metabolism, the O_2 reduction during photorespiration, and the Mehler reaction are maintained at a minimum level (Edwards and Baker, 1993). During coldness, the ratio between electrons transport and CO_2 assimilation has been enhanced, during an increase of the distribution of reductants towards other pathways comparatively with the CO_2 assimilation (Fryer *et al*., 1995; Massacci *et al*., 1995).

During this study the activity of the antioxidative enzymes has been investigated, under light stress, low temperatures and in the presence of certain photosynthetic inhibitors, during the state transitions of the exponentially *Mougeotia* cultures.

Material and Methods

The green alga *Mougeotia sp.* Agahrd (AICB 560) derives from The Collection of Algae Cultures of the Institute of Biological Research from Cluj-Napoca (AICB) (Dragoş *et al*., 1997). Strain AICB 560 was grown in Bold (BBM) nutritive solution, during continuous air stirring, constant 485 μmol. m^{-2}. s^{-1} light, at 22^{o}C. The cultivation period was of 18 days.

The light, inhibitors and low temperature treatment. The 4000 μmol. m^{-2}. s^{-1} PAR (photosynthetic active radiation) light intensity was applied for 60 minutes, at 0-2 ^{o}C, in the presence of certain specific inhibitors: 300 μM DCMU [3-(3, 4-dichlorophenyl)1, 1-dimethylurea], 2 μM FCCP (carbonyl cyanide-p-trifluormethoxy phenylhydrazone) and 5 mM iodoacetamide. The state transition of the photochemical activity was obtained using light with various composition for the photosystems excitation, namely: *state 1*: light 1 with a wavelength more than 700 nm, far-red, emitted by a combination of blue and red filters, at 470 μmol. m^{-2}. s^{-1} light, which activates the PS I and *state 2*: light 2 which is specific for PS II, emitted by a red filter, at 620 nm, with a 2300 μmol. m^{-2}. s^{-1} intensity.

The analysis of the antioxidative enzymes. The activity of the analyzed enzymes was measured at room temperature. The extraction medium contains 50 mM potassium phosphate buffer, pH=7. 8; 1 mM EDTA; 10mM mercaptoethanol and 2% PVP (polyvinylpyrolidone).

Ascorbate peroxidase (APX) was measured according to Leipner (1998). In the extraction medium 1 mM sodium ascorbate was added. The extraction medium contains 50 mM potassium phosphate buffer pH=7. 0; 200 μM DTPA (diethylenetriaminepentaacetic acid); 1 mM ascorbate and 250 μM H_2O_2 to which the enzymatic cell extract was added. The enzymatic activity was measured by decreasing the absorption at 290 nm using the extinction coefficient ε = 2. 8 mM^{-1}. cm^{-1}.

*Monodehydroascorbate reductase (*MDAR) was measured after modifying the Leipner method (1998). The extraction medium contains 50 mM potassium phosphate buffer pH=7. 8; 200 μM DTPA; 1 mM ascorbate; 0. 12 mM NADPH, 1 unit ascorbate oxidase and cell extract. The reaction begins after adding the ascorbate oxidase, and NADPH oxidation was measured by decreasing the absorption at 340 nm. The enzymatic activity was calculated using the extinction coefficient ε = 6. 2 mM^{-1}. cm^{-1}.

Dehydroascorbate reductase (DHAR) was measured after modifying the Leipner method (1998). The extraction medium contains 50 mM potassium phosphate buffer pH=7. 8; 200 μM DTPA; 2. 5 mM GSH; 1 mM dehydroascorbate and cell extract. The enzymatic activity was measured by monitoring the absorption enhancement at 265 nm using the extinction coefficient ε = 14 mM^{-1}. cm^{-1}.

Superoxyde dismutase (SOD) was measured after modifying the Leipner method (1998). The extraction medium contains 50 mM potassium phosphate buffer pH=7. 8; 200 μM DTPA; 13 mM methionine; 1 mM NBT (nitroblue tetrazolium); 4 mM riboflavin. After illuminating the mixture with a strong light, the cell extract was added and the absorption decrease at 560 nm was analyzed. The control does not contain cell extract and it is not illuminated. The amount of enzymatic extract that causes the absorption decrease with 50% was defined as 1 SOD unit.

Glutathione reductase (GR) was measured after modifying the Leipner method (1998). The extraction medium contains 50 mM potassium phosphate buffer pH=7. 8; 200 μM DTPA; 1 mM oxidized glutathione (GSSG) and 0, 12 mM NADPH. The enzymatic activity was measured the absorption decrease at 340 nm using the extinction coefficient ε = 6. 2 mM^{-1}. cm^{-1}.

Measuring the NAD(P)H-dehydrogenase. The reaction medium contained 3 ml cell extract in Tris-HCl buffer, 0. 12 mM NAD(P)H and 120 μM duroquinone. The activity of the NAD(P)H-dehydrogenase in darkness was spectrophotometrically measured by decreasing the absorption at 340 nm. The enzymatic activity was expressed in μmol/minute/mg chlorophyll a using the extinction coefficient ε = 6. 23 mM^{-1}. cm^{-1}.

The *lipid peroxidation* was measured according to Venisse et al. (2001). The algal material was extracted with 10% trichloroacetic acid. The reaction medium containing vegetal extract, 10% trichloroacetic acid and 0. 2% thiobarbituric acid was boiled for 30 minutes at 90^0 C and then was clarified by centrifugation. The TBARS amount (reactive species of thiobarbituric acid) was calculated based on the absorption at 600 nm, using the extinction coefficient ε = 155 mM^{-1}. cm^{-1}.

Results and discussion

In the photosynthetic organisms, the superoxide is formed through the univalent photoreduction of O_2 on the PS I reducing site in the thylakoid membrane when the photosynthesis is saturated by light (Miyake and Yokota, 2000; Schreiber and Neubauer, 1990). Superoxide dismutase (SOD) catalyses the superoxide radicals dismutation (O_2^-) producing molecular oxygen and hydrogen peroxide (H_2O_2), and thus the O_2^- concentration decreases, being the first link of the enzymatic system for the removal of the active oxygen.

Related to the control, the SOD activity has enhanced in almost all the variants (Fig. 1). *V_3* was an exception, where the anaerobiosis conditions induced by FCCP have lead to the decrease in the oxygen production, which is the main source of the oxygen reactive species. During s*tate 2*, the light 2 that is favorable for the PS II photochemistry, has lead to the intensification of SOD activity was a result of the intense oxygen production whose cellular utilization was blocked by the FCCP inhibitors and iodoacetamide. In *state 1*, light 1 which is favorable to PS I and in the presence of DCMU that blocks the transport of photosynthetic electrons from the PS II, leads to the utilization of the electrons from the PS I towards the de-activation of the reactive oxygen species. SOD and the enzymes that remove H_2O_2 intensify their activity in proportion with the light intensity that plays a regulator role of the antioxydative enzymes in chloroplasts (Cakmak and Marschner, 1992).

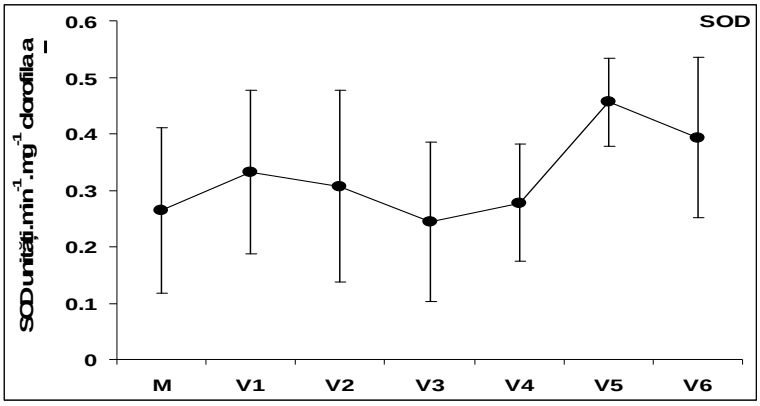

Fig. 1. Superoxide dismutase activity (SOD): M – control; V_1 –4000 µmol. m^{-2}. s^{-1} light; V_2 – 4000 µmol. m^{-2}. s^{-1} + T (0^0 – 2^0C); V_3 = 4000 µmol. m^{-2}. s^{-1} + T (0^0 – 2^0C) + FCCP; V_4 - 4000 µmol. m^{-2}. s^{-1} + T (0^0 – 2^0C) + FCCP + iodoacetamide; V_5 = *State 2*: light 2 (620 nm) + T (0^0 – 2^0C) + FCCP + iodoacetamide; V_6 = *State 1:* light 1 (> 700 nm) + T (0^0 – 2^0C) + FCCP + iodoacetamide + DCMU

By blocking the PS II, the cyclic electrons flow around PS I performs at the maximum rate of photosynthesis inducing the ATP synthesis, which also implies the cytochrome b_6f (Joliot and Joliot, 2002). The photogeneration of toxic oxygen species is intensified when the plants are exposed to certain stress factors that block the utilization of the absorbed light energy for the CO_2 fixation (Asada, 2006). The damages produced by the low temperature together with the light can be quantified through the active oxygen species. The acclimatization for coldness leads to some changes in the antioxidants isoenzymes composition (Pinhero *et al*., 1997).

The hydrogen peroxide that is formed from superoxide by the disproportionation catalyzed by superoxyde dismutase, is reduced to water through ascorbate peroxidase using the ascorbate as an electrons donor (Mittler and Zilinskas, 1991).

The oxidized ascorbate is reduced to ascorbate by ferredoxine, monodehydroascorbate reductase and dehydroascorbate reductase. The ascorbate peroxidase (APX) activity is located in the cytosol and in the chloroplasts (Asada, 1999). The chloroplastic and cytosolic isoenzymes unlike each other due to the large lability of the chloroplastic form in the absence of ascorbate.

The activity of the ascorbate peroxidase was intensified in all the variants (Fig. 2). Together with the strong light, the low temperature and the anaerobiosis conditions induce the activation of APX. The amount of APX increases in strong light and in the presence of methyl-viologen (Yoshimura *et al*., 2000).

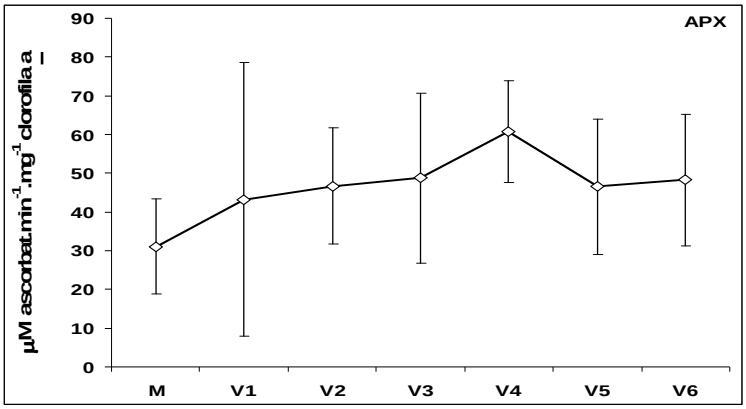

Fig. 2. Ascorbate peroxidase activity (APX) (see Fig. 1 legend)

The catalytic removal of hydrogen peroxide through APX produces monodehydroascorbate (MDA), which decomposes in a non-enzymatic way through spontaneous disproportionation in ascorbate and dehydroascorbate or by univalent oxidation of ascorbate in enzymatic reactions. In chloroplast stroma, MDA is enzymatically reduced to ascorbate by monodehydroascorbate reductase

(MDAR) that uses NADH and NADPH as electron donors. MDA may be reduced by the PS I in the ferredoxin pathway, also (Miyake and Asada, 1994). The cytochrome b_6f and its reductase participation in MDA reduction was noticed by *Hara* and *Minakami* (1971).

Monodehydroascorbate reductase is different form NADH-dehydrogenase and other enzymes due its molecular weight, amino acid composition and its specificity for electron donors and acceptors. MDA reductase is localized in the chloroplast stroma (Hossain and Asada, 1985).

The monodehydroascorbate reductase activity is reduced in almost all the variants (Fig. 3). An exception is noticed when the enzyme activity was tested in anaerobic conditions created by the FCCP presence, when the enzyme activity was more intense comparative with the control (V_3). In the DCMU presence, in *state 1*, the photochemical activity is sustained only by PS I where the reactive oxygen species are generated, which imply the intensification of the antioxidative enzymes activity that can be found in the control (V_6).

The importance of Mehler reaction in removing the peroxide excess was demonstrated by the increment of APX activity as a response to the environmental stress factors. The cell recovery of ascorbate is based on direct reduction of MDA radical to ascorbate by the MDA reductase, using the NADPH as electron donor. Simultaneous, the MDA radical is spontaneous transformed in ascorbate and DHA – in this case DHA is reduced by DHA reductase and GSH reductase using the GSH and NADPH as electron donors. Thus, the presence of ascorbate/glutathione recovery system was studied more in chloroplasts, but this kind of enzymes exists in cytosol also. Many enzymes from Calvin cycle are rapidly inactivated if the hydrogen peroxide concentration is not preserved at low levels by the APX catalytic activity.

The dehydroascorbate reductase activity is significantly reduced in the presence of high light and low temperature and in conditions of anaerobiosis and photosystems state transition as against the control values (Fig. 3). The decrement of dehydroascorbate reductase is due to a reduced amount of dehydroascorbate which was consumed in other metabolic pathways. DHAR and GR were severely inhibited using a 5^oC temperature value (Anderson *et al*., 1995).

Glutathione reductase (GR) is a flavoprotein that catalyzes the oxidized glutathione reduction (GSSG to GSH) in NADPH presence as an electron acceptor. GR together with APX and DHAR constitute the chloroplast system for H_2O_2 removing. The GR activity is localized mainly in the chloroplast and it is involved in glutathione recovery (Karpinski *et al*., 1993; Foyer and Halliwell, 1976).

The GR activity increased in conditions of intense light and low temperature as against the control values (Fig. 4, V_1, V_2). The enzyme activity decreased significantly in anaerobic conditions induced by FCCP and iodoacetamide (V_3, V_4). The GR potential activity of the photosystems was reduced in *state 1* and *state 2*.

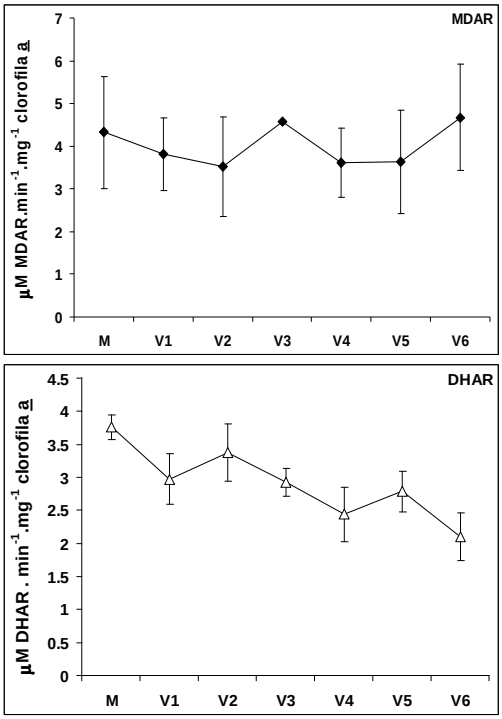

Fig. 3. Monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) activity (see Fig. 1 legend)

NADPH-dehydrogenase is an oxidoreductase that acts on NADPH, the substrate which intervenes in CO_2 fixing mechanism. The stress factors produce reactive oxygen species based on the NADPH-oxidase activity intensification (Rao *et al*., 1996) that was observed in many plant species (Moller and Lim, 1986). CO_2 fixation through oxygenic photosynthesis depends on NADPH and ATP generation by the electron transport stimulated by light, from the water to $NADP^+$. In anaerobic conditions, ATP was synthesized without an NADP/NADPH ratio change due to the cyclic electrons flow. In anaerobic conditions, ATP increased and NADPH decreased.

The O_2 evolution was stimulated by the inhibitors adding. These observations suggest that the photosynthetic generation of reductive equivalents limits the photosynthetic assimilation (Forti *et al*., 2003).

The obtained results as for the NADPH-dehydrogenase activity showed an activity inhibition in all experimental conditions (Fig. 5). Mehler reaction and the cyclic electrons flow around PS I photosystem may contribute to the increment of photosynthetic ATP/NADPH ratio. In aerobic conditions, the NADPH generation

rate, showed by the NADPH-dehydrogenase activity that uses NADPH as enzymatic substrate, is an important process in photosynthetic assimilation. The NADPH-dehydrogenase complex is involved in the nonphotochemical reduction of the plastoquinone in darkness, after preillumination (Cournac *et al*., 2000).

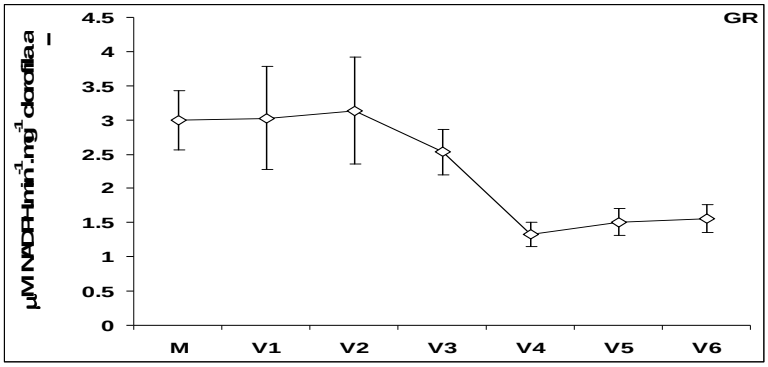

Fig. 4. Glutathione reductase (GR) activity (see Fig. 1 legend)

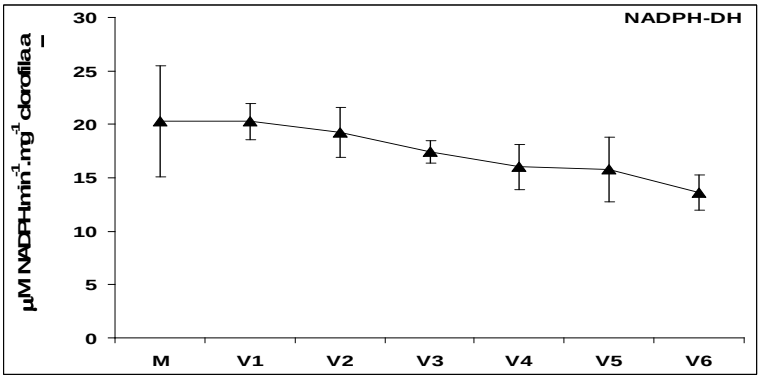

Fig. 5. NADPH-dehydrogenase activity (see Fig. 1 legend)

Lipid peroxidation through lipoxygenase generates singlet oxygen and radicals as anionic superoxide. Lipoxygenase catalyzes the reaction between O_2 with free, polyunsaturated fatty acids producing hydroperoxides of conjugated lipids. The lipoxygenase activity increment is correlated with the high MDA content, a product of lipid peroxidation. The increment of lipid peroxidation is showed by the increment of lipoxygenase activity (Fryer *et al*., 1998). Lipid peroxidation determines the alteration of the membrane integrity (Venisse *et al*., 2001).

The lipid peroxidation inducement was examined by detecting the accumulation of reactive thiobarbituric species (TBARS) as a consequence of thiobarbituric acid reaction in trichloroacetic acid (Fig. 6). Lipid peroxidation inducement was slightly diminished comparative with the control sample (V_1), under intense light and it was raised in conditions of low temperature, probably because of the increment of oxidative damages (V_2).

Lipid peroxidation raised significantly comparative with the control in the presence of light 2, in *state 2*. The lipid peroxidation activity increased two times in *state 1*, if the DCMU photosynthetic inhibitor was added (V_6).

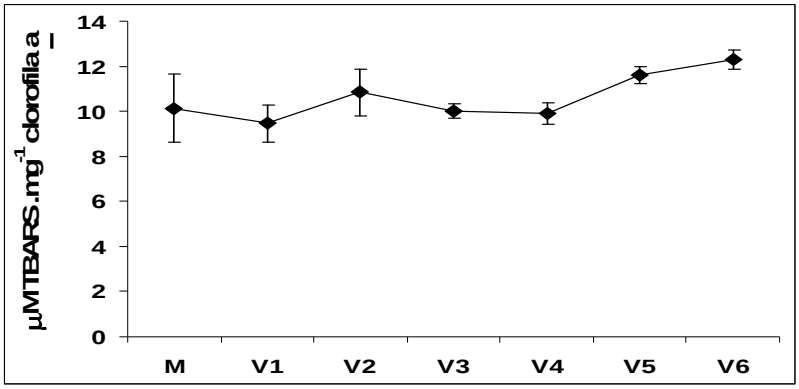

Fig. 6. Lipid peroxidation activity (see Fig. 1 legend)

The activity of antioxidative enzymes, induced more or less by the photochemical state transition process, certifies that the relative flux of O_2 reductive photosynthetic equivalents through the Mehler reaction is rather high. The maintenance of the electron flow in thylakoids in conditions of enough amounts of $NADP^+$ as electron acceptor is essential for the chloroplast protection from photooxidative stress. The cyclic electrons flow and the water-water cycle around PS I am involved in this process (Rizhsky *et al*., 2003). The photogenetion of reactive oxygen species may intensify when the plant are exposed to intense light in combination with stress factors as low temperature that limit the usage of light energy absorbed for CO_2 fixation in photosynthesis. These conditions determine the limitation of disposable $NADP^+$ used to accept the electrons from the PS I and so, O_2 is activated (Cakmak and Marschner, 1992).

Conclusions

1. The SOD and glutathione reductase activity increased in almost all the variants in anaerobic conditions induced by the FCCP, which determined a decrement in oxygen synthesis, the main source for reactive oxygen species. By blocking the PS II,

the cyclic electrons flow around PS I functions with a maximum rate of photosynthesis, inducing the ATP producing, a process that imply the complex b_6f too.

2. The ascorbate peroxidase activity raised in conditions of high light, low temperature and anaerobiosis.

3. The monodehydroascorbate reductase activity decreased, excepting for the anaerobic conditions.

4. The dehydroascorbate reductase and NADPH-dehydrogenase activity was significantly reduced in the presence of high light, low temperature and in conditions of anaerobiosis and photosystems state transition also.

5. Lipid peroxidation intensified in conditions of high light and low temperature, probably due to the increment of oxidative degradation. Lipid peroxidation increased two times if the photosynthetic inhibitor DCMU was added in *state 1*.

Acknowledgments. The present research was supported by *CERES Programme* nr. 54/2006.

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THE KINETICS OF CHLOROPHYLL FLUORESCENCE INDUCEMENT IN STATE TRANSITION IN *MOUGEOTIA* GREEN ALGA, STRAIN AICB 560

VICTOR BERCEA[1], ADRIANA BICA[1, 2], NICOLAE DRAGOŞ[1, 2]

SUMMARY. The photochemical kinetics of the photosystems, based on the evolution of chlorophyll fluorescence in state transitions and in the presence of some photosynthetic inhibitors was studied in *Mougeotia* cells. *The state 1* was induced by excitation with far-red light *1* (720 nm) and light *2* (665 nm) for the *state 2*, respectively, in aerobic conditions and in the presence of 2 µM FCCP. The reaction centers ratio, the photochemical activity and the plastoquinone reduction increased in *state 1*, in the presence of 300 µM DCMU, 20 µM DBMIB and 230 mM chloramphenicol. The oxidation state of plastoquinone and the usage of excitation light in photochemistry increased without a non-photochemical dissipation of the excitation in *state 2*. DCMU reduces the photochemical efficiency and the yield because of the photoinhibition. The inhibitors combination, which promotes PS I oxidation, induced the plastoquinone reduction state due to the PS I electrons. In *state 1-state 2* transition, light 2 contributes to increasing of the open reaction centers ratio and so, the plastoquinone may function in high oxidative conditions. The *state 2-state 1* transition increases the ratio of the open reaction centers.

KEYWORDS: quenching coefficients, state 1, state 2, state 1 – state 2 transition, state 2 –state 1 transition

Introduction

The state transitions has been described as a chromatic adaptation of short duration, which allows the plant and algae to respond to the spectral quality of light, through the variation of the PS II and I light absorption. The process is mediated by the transferring of a protein component part of LCH II antenna complex, from the PS II to PS I, in *state 1-state 2* transition, which balances the excitation energy redistribution between photosystems. The cytochrome b_6f complex regulates the state transitions (Finazzi, 2005; Vallon *et al.*, 1991).

The LCH II – PS I migration through lateral diffusion (*state 1-state 2* transition) is due to the LCH phosphorylation mediated by a protein-kinase bound to the thylakoid membrane which is activated when plastoquinone is reduced

[1] *Institute of Biological Research, 48 Republicii Street, 400015/Cluj-Napoca, Romania E-mail: bercea_victor@yahoo.com*

[2] *Faculty of Biology and Geology, Babeş-Bolyai University, 5-7 Clinicilor Street, 400006/Cluj-Napoca, Romania*

(Allen, 1992; Vener *et al*., 1997). In oxidative conditions, the kinase is inhibited and LCH II dephosphorylation (*state 2-state 1* transition) is achieved by a phosphatase, which is supposed to be constitutively active (Cannani *et al*., 1984; Finazzi *et al*., 2001a; 2002; Forti and Caldiroli, 2005; Williams and Allen, 1987). The cytochrome b_6f complex is accumulated in the thylakoid stromal lamella in *state 2* (Finazzi et al., 2002). *State 2* represents the structural conditions which uses the excitation energy in PS I photochemistry, so the electrons cyclic transport around the PS I predominates the electron linear flux which implies both photosystems (Finazzi *et al*., 2001 a).

Finazzi *et al*. (2001a) have established that s*tate 1* is obtained through incubation of the cells in the dark under strong agitation, whereas *state 2* is obtained through dark incubation in anaerobic conditions obtained by argon bubbling. Incubation of the cells in conditions that promote the *state 2* – anaerobic or aerobic and FCCP – generates an electron source through cytochrome b_6f complex, different of that produced by the PS II and it is active when *state 1-state 2* transition does not produce. The reactivation of the electron linear flux between PS II and PS I depends on *state 2 – state 1* commutation and is indicated by a concomitant rising of the fluorescence (F_m).

Two hypotheses were proposed explaining the phenomenon. LCH II migration may be caused by protein conformational changes in phosphorylation process (Nilsson, 1997), playing an important role in P-LHC – PS I bounding. An alternative hypothesis proposes the electrostatic repellence generated by the increasing of the negative charges in thylakoids that determines the detachment of the P-LCH II from PS II.

In the present paper, we studied the photosystems photochemical kinetics based on chlorophyll fluorescence evolution in state transitions, in the presence of some inhibitors of photosynthetic electrons transport chain.

Materials and Methods

The green alga *Mougeotia sp*. Agardh (AICB 560) derived from the Algae Culture Collection of the Institute of Biological Research from Cluj-Napoca (AICB) (Dragoş *et al*., 1997). The alga was grown in Bold nutritive solution (BBM), under continuous air agitation, illumination with 630 µmol. m^{-2}. $s^{-1}s^{-1}$, at 22^{o}C. The period of growing was 23 days.

The quenching analysis of fluorescence. The induction curves of chlorophyll fluorescence with quenching analysis have been recorded in *state 1* and *state 2*, *state 1 – state 2* transition or *state 2-state 1* transition, using 650 nm measuring light and saturation pulse mode as follows:

-*state 1* – excitation with far-red light (light *1*, 720 nm) for 30 seconds to excite the PS I, then applying the saturation pulse

-*state 2* – excitation with actinic light (light *2*, 665 nm) for 5 seconds to excite the PS II, followed by applying the saturation pulse

-*state 1 – state 2 transition*: excitation with far-red light (light *1*) for 15 seconds, then excitation with actinic light for 2 seconds (light *2*)

-*state 2 – state1 transition*: excitation with actinic light (light *2*) for 2 seconds, then excitation with far-red light (light *1*) for 15 seconds

The photosynthetic inhibitors used: FCCP (carbonylcyanide-p-trifluoromethoxyphenylhydrazone), an uncoupling which causes the transition to *state 2* by decreasing the ATP and the membrane potential (V_1); DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimetylurea) inhibits the electrons transfer between Q_A and Q_B (V_2); FCCP+DCMU (V_3); FCCP+DBMIB (DBMIB (2, 5-dibromo-3-methyl-6-isopropylbenzoqinone)) inhibit the photosynthetic electrons transfer from plastoquinone to cytochrome b_6f complex (V_4); FCCP+chloramphenicol (chloramphenicol inhibits the protein synthesis) (V_5). The algae suspensions were incubated for an hour, in darkness, in the presence of inhibitors, and then the state transition was induced. The chlorophyll fluorescence was measured with PAM-210 fluorimeter according to Schreiber *et al*. (1986).

Results and discussion

On short time, the plants response to the light quality represents the s*tate1 – state 2 transition* mechanism (*state 1* induced by the far-red light (>700 nm) preferential absorbed by PS I; *state 2* induced by the red light (<680 nm) preferential absorbed by PS II), fact shown by the redox changes of the plastoquinone and cytochrome b_6f complex (Su and Shen, 2003).

The exposure to short wavelength light determines the adaptation of the photosynthetic apparatus to *state 2* through energy redistribution in favor of PS I, to balance the excitation between photosystems. This process is reversible in the presence of far-red light obtaining the *state 1*, when the short light excites mainly PS II photosystem (Veeranjaneyulu and Leblanc, 1994).

The kinetics of chlorophyll fluorescence in *state 1* (excitation with far–red, 720 nm) shown a decreasing of $F`_m$, photochemical efficiency ($q_p$) and an increasing of $F`_0$ in control sample (Fig. 1, M). The open reaction centers increased, showing the oxidation state of plastoquinone. $F`_m$, $F`_0$, photochemical efficiency (q_p) and yield (Y) increased in the presence of 2 µM FCCP (Fig. 1, V_1). The reaction centers ratio, the photochemical activity of both photosystems and the reduction state of plastoquinone increased. The induction of oxidation conditions to plastoquinone, in presence of 300 µM DCMU has produced an alternation in increment and decrement of $F`_m$, $F`_0$, with a slightly increment in the yield and a high value for the photochemical efficiency (V_2).

DCMU inhibits the Q_B reduction of PS II, so the plastoquinone oxidation produced in light presence and the *state 1* is achieved. In *state 1*, the majority of antenna proteins are associated with PS I (Takahashi *et al*., 2006). The FCCP+DCMU mixture determined a high value for F_m, a small value for $F`_0$, $F`_m$, q_P and an oscillation for the yield (V_3).

In presence of FCCP and 20 nM DBMIB, F_m, F'_m have increased, while F'_0, q_P have decreased, and the yield has oscillated (*V4*). The rise of the closed reaction centers induced the plastoquinone reduction state and reduced photochemical activity. The FCCP+230 µM chloramphenicol combination determined the increment of F_m, $F`_m$, $F`_0$ and decreased PS II efficiency (q_p) and yield of photosynthesis (*V5*). The efficiency of energy harvest through the open centers was influenced by the internal structural changes of photosystems.

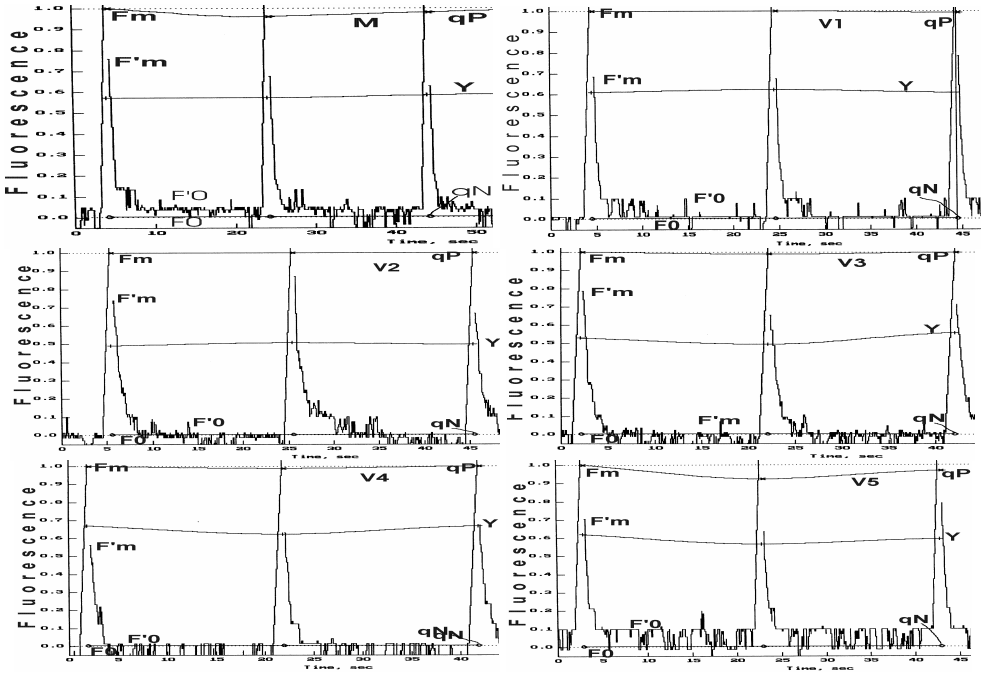

Fig. 1. The induction curves and quenching analysis of algal suspension in *state 1*. M – control; V_1- 2 µM FCCP; V_2- 300 µM DCMU; V_3- 2 µM FCCP+300 µM DCMU; V_4- 2 µM FCCP+20 mM DBMIB; V_5- 2 µM FCCP+230 µM chloramphenicol; F_o-minimal fluorescence; F_m-maximal fluorescence; F'_m, F'_0- fluorescence after illumination; Y-yield; q_P- photochemical quenching; q_N- non-photochemical quenching.

The fluorescence kinetics in *state 2* (excitation with light *2*, 665 nm) produced F_m decrement, $F`_m$ and F_0 increment and yield (Y) and photochemical efficiency (q_p) oscillated in control sample, after applying three saturation pulses (Fig. 2, M). The quenching analysis revealed the photochemical coefficient (q_p) decrement and the non-photochemical quenching (q_N) increment. In FCCP presence, F_m and $F`_m$ decreased, $F`_0$ increased and q_p raised at a maximum value explaining the light usage in photochemistry only, without a non-photochemical dissipation of the excitation (small value for q_N), stimulating the plastoquinone oxidation state (Fig. 2, *V1*).

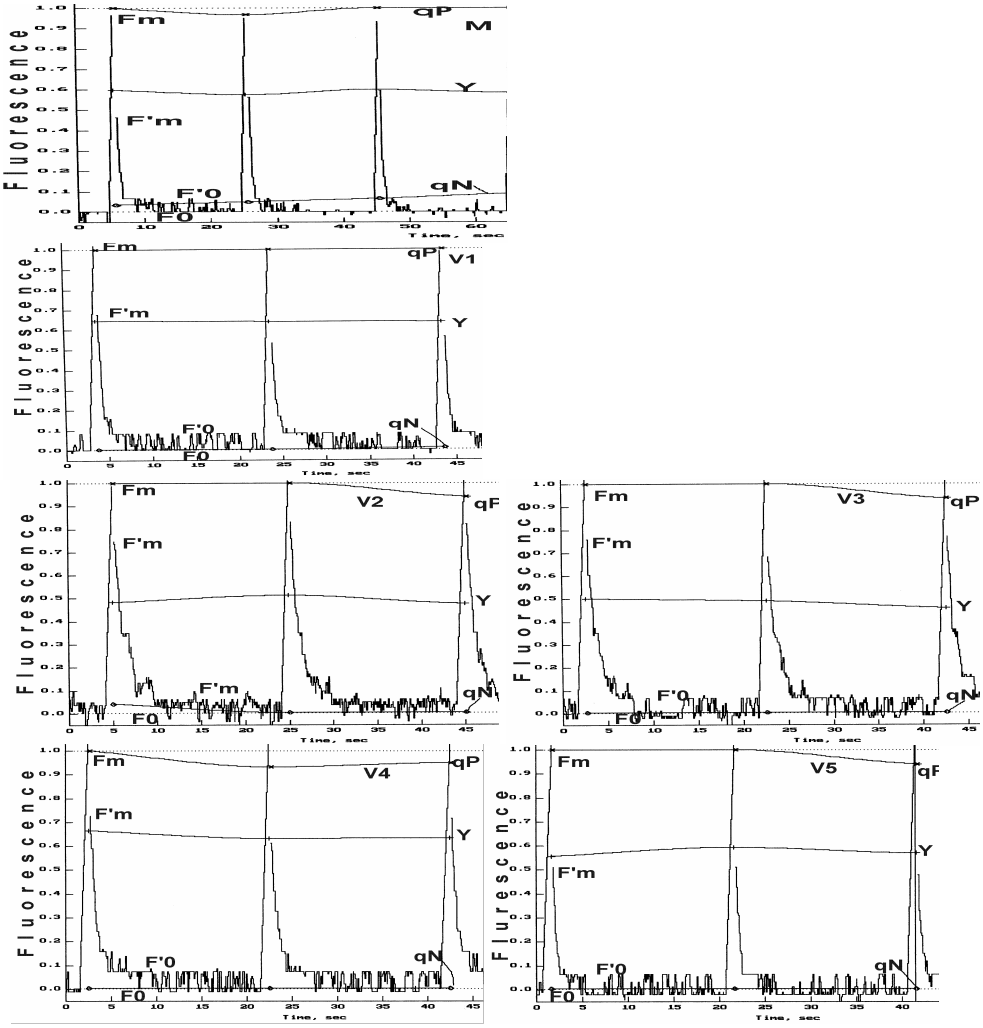

Fig. 2. The induction curves and quenching analysis of algal suspension in *state 2*. M – control; V_1- 2 μM FCCP; V_2- 300 μM DCMU; V_3- 2 μM FCCP+300 μM DCMU; V_4- 2 μM FCCP+20 mM DBMIB; V_5- 2 μM FCCP+230 μM chloramphenicol; F_o-minimal fluorescence; F_m-maximal fluorescence; F'_m, F'_0- fluorescence after illumination; Y-yield; q_P- photochemical quenching; q_N- non-photochemical quenching.

In DCMU presence, when the plastoquinone oxidation is induced, the fluorescence parameter F_0, F_m, F'_0, F'_m were increased after applying three saturation pulses which determined a decrement of photochemical efficiency (q_p), yield and non-photochemical quenching (q_N) due to the achievement of photoinhibition (V_2). The FCCP+DCMU mixture determined an F'_0, F'_m increment. The photochemical

efficiency (q_p) and the yield decreased and the non-photochemical dissipation of excitation was insignificant (V_3). In this case, PS I electrons are responsible for the reduction of the plastoquinone. The FCCP+DBMIB combination, that facilitates the oxidation conditions for PS I, induced the rising of basal fluorescence and an F_m, photochemical efficiency (q_p) and yield decrement, so the plastoquinone passed in reduced state (V_4). In FCCP+chloramphenicol presence, F_0, F'_0, F_m increased and F'_m, the photochemical efficiency (q_P) and the yield decreased, so the photochemical activity of the photosynthetic electrons linear flow was diminished (V_5).

Light *2* that excites mainly the PS II induces a PS I high sensitivity while the transfer to the far-red light that excites PS I produces a PS II high sensitivity (Wollman, 2001). In both states an F_m and F_0 quenching was observed and so the F_v/F_m ratio remained constant. *State 1-state 2 transition* produced changes in the PS II internal organization (Hodges and Barber, 1983).

The kinetics of chlorophyll fluorescence in *state 1 – state 2 transition* is shown in Fig. 3.

In the control sample, F_0, F'_0 increased and F'_m, the photochemical efficiency (q_P) and the yield oscillated (M), creating oxidation conditions for the plastoquinone. In FCCP presence F_0, F'_0 and yield raised while the photochemical efficiency decreased (V_1). The increment of the open reaction centers and the reducing of the photochemical efficiency induced the plastoquinone reduction state.

In the DCMU presence, F'_0 decreased and F'_m increased, allowing the plastoquinone functioning in oxidative conditions (V_2). The FCCP+DCMU mixture produced an F'_m and yield increment and an F'_0 decrement. The high ratio of the open reaction centers stimulated the PS II electron transport and the plastoquinone oxidative state (V_3). The FCCP+DBMIB mixture produced a slightly rise of F_m, F'_0 and yield and F'_m fluctuated (V_4). DBMIB inhibited state transitions after preillumination of the cell (Finazzi *et al*., 2001 b). Chloramphenicol and FCCP determined a variation of the maximum fluorescence and a slightly rise of the yield and F'_0 (V_5). In the presence of the used inhibitors, light *2 in state 1 – state 2 transition* contributes to the maintenance of a high ratio of the open reaction centers, making possible the plastoquinone functioning in high oxidative conditions. In these conditions, the non-photochemical dissipation of the excitation energy was reduced at minimum.

The kinetics of chlorophyll fluorescence in *state 2 – state 1 transition* is shown in Fig. 4. In the control sample, F'_0, F'_m, the photochemical efficiency and the yield became fluctuant. In FCCP presence, F'_m, F'_0 decreased and the yield and the photochemical efficiency raised. F_m kept constant at maximum values (V_1). The increment of the open reaction centers shows the usage of the excitation energy in photochemistry only, inducing the reduction state in PS II and plastoquinone.

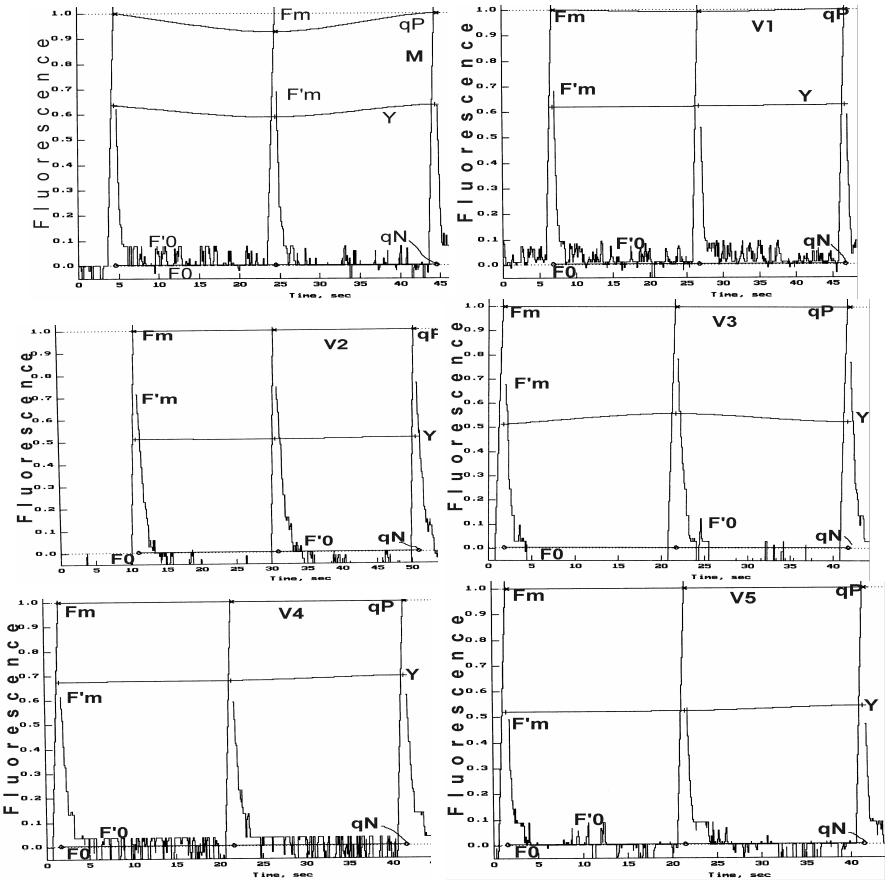

Fig. 3. The induction curves and the quenching analysis of algal suspension in state 1-state 2 transition. M – control; V_1- 2 μM FCCP; V_2- 300 μM DCMU; V_3- 2 μM FCCP+300 μM DCMU; V_4- 2 μM FCCP+20 mM DBMIB; V_5- 2 μM FCCP+230 μM chloramphenicol; F_o- minimal fluorescence; F_m-maximal fluorescence; F'_m, F'_0- fluorescence after illumination; Y-yield; q_P- photochemical quenching; q_N- non-photochemical quenching.

In DCMU presence, F'_m, the quantic efficiency (q_P) and the yield decreased and F_m and F'_0 increased (V_2). The efficiency of energy harvest through the open reaction centers diminished in conditions of a high plastoquinone reduction state due to the PS I activity. The FCCP+DCMU mixture produced an F'_0 decrement, a slightly increment in yield and F_m and F'_m fluctuation (V_3). In FCCP+DBMIB presence F'_m, F'_0 increased and the yield decreased (V_4). In FCCP+chloramphenicol the fluorescence parameters remained constant with the exception of F'_m şi F'_0 (V_5). In *state 2 – state 1 transition*, the high ratio of the open reaction centers demonstrates the effectiveness of excitation energy usage in photochemistry and the PS II reduction state inducement,

though the photochemical efficiency is diminished. The non-photochemical dissipation of the excitation energy was not observed in none of the experimental samples.

The parameters of the fluorescence kinetics and in their short-time dynamic were correlated with the evolution of the long-time fluorescence parameters, in condition of light exposure and they shown approximately the same photochemical activity in both photosystems according to the intensity and quality of the excitation light (Bercea *et al*., 2008).

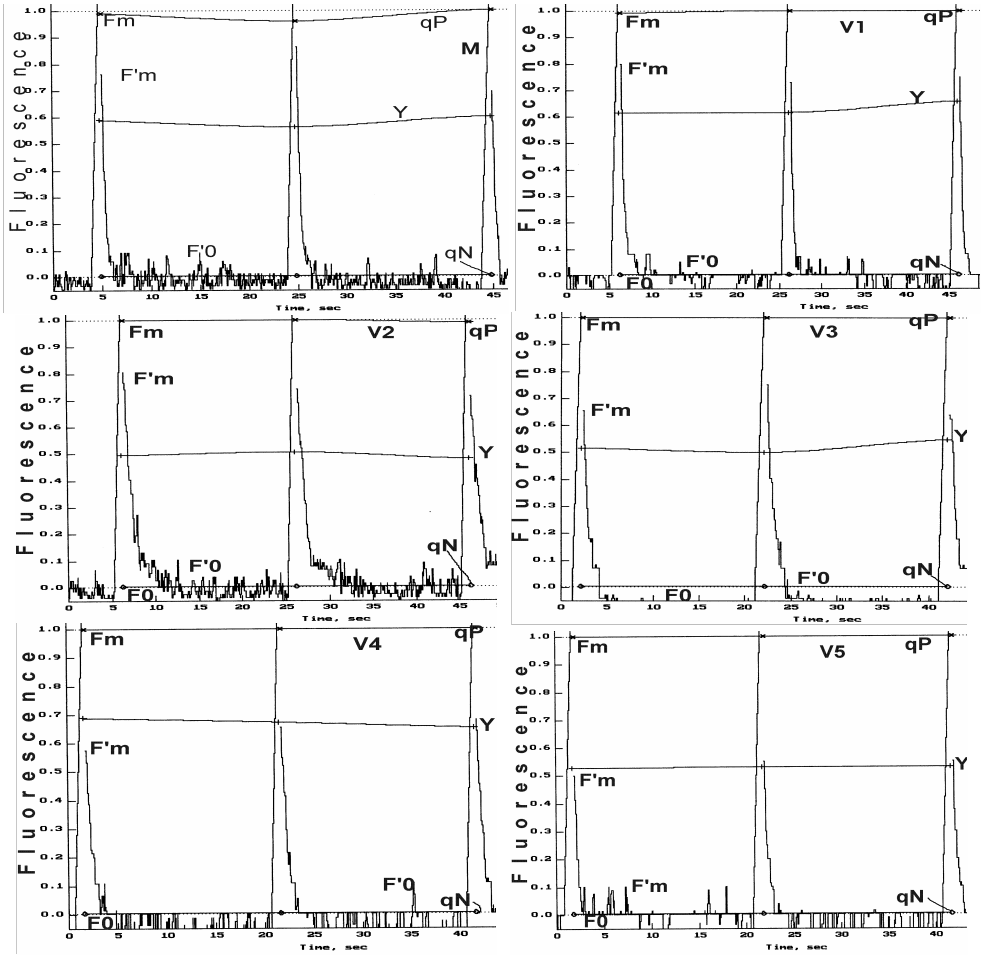

Fig. 4. Induction curves and the quenching analysis of algal suspension in *state 2 – state 1 transition*. M – control; V_1- 2 μM FCCP; V_2- 300 μM DCMU; V_3- 2 μM FCCP+300 μM DCMU; V_4- 2 μM FCCP+20 mM DBMIB; V_5- 2 μM FCCP+230 μM chloramphenicol; F_o- minimal fluorescence; F_m-maximal fluorescence; F'_m, F'_0- fluorescence after illumination; Y-yield; q_P- photochemical quenching; q_N- non photochemical quenching.

Conclusions

1. The reaction centers ratio, the photochemical activity in both photosystems and the plastoquinone reduction state increased in *state 1* (excitation with far-red light, 720 nm), in the presence of photosynthetic inhibitors. DCMU inhibits Q_B reduction in PS II, so the oxidation of plastoquinone produces in condition of light exposure and *state 1* is achieved. The increment of the plastoquinone reduction state is due to the imbalance created in the photochemical activity of the photosystems.

2. In *state 2* (excitation with light 2, 665 nm), in FCCP presence, the excitation energy usage raised in photochemistry only, without a non-photochemical dissipation of the excitation and the plastoquinone oxidation state increased. DCMU reduces the photochemical efficiency and the yield because of the photoinhibition achievement. The inhibitors mixture, which promotes PS I oxidation, induced the plastoquinone reduction state due to the PS I electrons. The photochemical efficiency and the yield decreased, diminishing the photochemical activity of the linear flow of photosynthetic electrons.

3. In the presence of these inhibitors, in *state 1 – state 2* transition, the light *2* maintains a high ratio of open reaction centers and so the plastoquinone functions in high oxidative conditions. In these conditions the non-photochemical dissipation of the excitation energy is reduced to a minimum value.

4. In *state 2 – state 1* transition, the high ration of the open reaction centers proves the usage effectiveness of the excitation energy in photochemistry and the inducement of the PS II reduction state, though the photochemical efficiency is diminished.

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A MODEL FOR POPULATION WITH RESPECT TO THE IMMIGRATION

MOHAMMAD REZA MOLAEI[1], MAHBOOBEH MOHAMMADHASANI[1]

SUMMARY. In this paper we introduce a model for population with respect to the per capita birth and with considering the number of people who immigrate from the society and to the society. In this model the per capita birth and the immigrant percentages are time dependent. Then we get conditions which make the number of all age groups fixed.

KEYWORDS: per capita birth; age group structure; hyperbolic fixed point; immigration

Introduction

Population and studying about it is one of the most important issues of society of human beings. In this way each subject about population is considerable (Dordea and Coman, 2006, Hoseini Anvari and Molaei,2006).

In the mathematical direction, age structure model (Farkas, 2001) is one of the most important models. In this model the only age groups who participating in reproduction are matures. Moreover per capita birth and death rate are time dependent. In this model the population is divided into n age groups. All the classifications have the same long. For example for human population, a lifespan of 150 years is broken down into 5-year groups. It means $n = 30$ age groups.

We would like to expend this model with paying attention to immigration as an important time dependent phenomenon which affects on population. For this goal we introduce two percentages for immigration, one for those who immigrate from the society and one for people who immigrate to the society. It is remarkable that per capita birth for people who immigrate to the society is different from per capita birth inside the society. In section 3 we get equations which determine the fixed points of the semi-dynamical systems created by the model. Moreover we present some conditions for the hyperbolicity of fixed points.

[1] *Department of Mathematics, Shahid Bahonar University of Kerman, 76169-14111, Kerman, Iran; E-mails: mrmolaei@mail.uk.ac.ir; mahboobehmh2003@yahoo.com*

The model

In this section we introduce some factors that are related to increasing and decreasing of population, especially the number of that.Let divide the population into n age groups. The per capita birth of the k^{th} age group at time j is denoted by $b_k(j)$. It is considerable that per capita birth of any group is time dependent and $b_k(j)$ is fertility of k^{th} age group at time j.

$x_k(j)$ is applied for distinguishing the number of individuals in the k^{th} group at time j. Then k^{th} group affects on the number of population with $b_k(j)x_k(j)$.

The mortality of those are in k^{th} age group at time j is denoted by $d_k(j)$. In the other word the percentage of those in k^{th} age group at time j who survive to form age group $k+1$ at time $j+1$ is $0 \le s_k(j+1) = 1 - d_k(j)$.

$e_k(j)$ is the percentage of those immigrate from society and also leave k^{th} age group at time j (just before leaving society they have been in k^{th} age group at time j).

However $x_k(j)s_k(j)$ are the number of those survive in k^{th} age group at time j and $x_k(j)s_k(j)e_k(j)$ are those who survive and also immigrate from the society, from k^{th} age group at time j.

Finally $x_k(j)s_k(j)e_k(j)b_k(j)$ are those are born at time j by the people who immigrate from the society (we have this assumption that the people who immigrate, take their offspring with themselves).
Similarly the percentage of those immigrate to the society and increase the number of that and enter into k^{th} age group at time j is denoted by $c_k(j)$, (in this case the percentage is considered with respect to the number of those are in k^{th} age group at time j. For example if the number of people in k^{th} age group at time j is 100 and the number of individuals who immigrate to the society and enter into k^{th} age group at time j and increase the number of that is 4 then we say $c_k(j) = \frac{4}{100}$). Assume per capita birth of those immigrate to the society and are placed in k^{th} age group at time j is denoted by $b_k^{'}(j)$. So that $x_k(j)c_k(j)b_k^{'}(j)$ is those who are born by the immigrants that enter to the society.

Then for given $j \in \{0,1,2,...\}$

$$x_1(j+1) = \sum_{k=1}^{n} \left(b_k(j) x_k(j) - x_k(j) s_k(j) e_k(j) b_k(j) + x_k(j) c_k(j) b_k^{'}(j) \right) =$$

$$\sum_{k=1}^{n} [b_k(j)[1 - s_k(j) e_k(j)] + c_k(j) b_k^{'}(j)] x_k(j) = \sum_{k=1}^{n} \alpha_k(j) x_k(j)$$

and for $k \geq 1$

$$x_{k+1}(j+1) = s_k(j) x_k(j) - x_k(j) e_k(j) + x_k(j) c_k(j) = [s_k(j) - e_k(j) + c_k(j)] x_k(j)$$

$$= \beta_k(j) x_k(j)$$

where

$\alpha_k(j) = b_k(j)[1 - s_k(j) e_k(j)] + c_k(j) b_k^{'}(j)$ and

$\beta_k(j) = s_k(j) - e_k(j) + c_k(j)$.

This model is applied for the population of a society with considering immigration as a time dependent phenomenon.

If $x(j) = (x_1(j), x_2(j), ..., x_n(j))$ for all $j = 0,1,2,...$ then we have the following generalization of Leslie matrix (Leslie, 1945, Leslie, 1948).

$$L(j) = \begin{bmatrix} \alpha_1(j) & \alpha_2(j) & . & . & . & \alpha_n(j) \\ \beta_1(j) & 0 & 0 & 0 & 0 & 0 \\ 0 & \beta_2(j) & 0 & . & . & 0 \\ 0 & .. & . & . & . & 0 \\ . & . & . & . & . & 0 \\ 0 & 0 & . & . & \beta_{n-1}(j) & 0 \end{bmatrix} .$$

We also have $x(j+1) = L(j) x(j)$ for all $j = 0,1,2,....$

Thus $x(j) = (L(j))^j x(0)$ for $j = 0,1,2,....$

Since $\det L(j) = (-1)^{n-1} \alpha_n(j) \beta_1(j) \beta_2(j) ... \beta_{n-1}(j)$, then under the conditions $\alpha_n(j) \neq 0$ and $\beta_k(j) \neq 0$ for all $k = 1,2,...,n-1$ we have $x(j) = (L(j))^{-j} x(j+1)$ for all $j = 0,1,2,...$ .then $x(0) = (L(j))^{-j} x(j)$ for all $j \in \{0,1,2,...\}$.

Fixed Points

In this section we want to find some conditions which make the number of population fixed. We define the following mapping

$\psi_j : R^n \to R^n$

$x(j) \mapsto x(j+1)$ where j=0,1,2,.. .

With respect to $x(j+1) = L(j)x(j)$ $,for\ \ all\ \ j = 0,1,2,...$ we have

$\psi_j^m(x(j)) = L(j+m-1)L(j+m-2)...L(j)x(j) = x(j+m),$

where m is a natural number.

Thus $\psi_j^t o \psi_j^m(x(j)) = \psi_j^t(x(j+m)) = x(j+m+t) = \psi_j^{t+m}(x(j))$.

So $\psi_j^t o \psi_j^m = \psi_j^{t+m}$ for all natural numbers $t\ \ and\ \ m$.

Thus $\{\{R^m, \{\psi_j^m, m \in N\}\}, j = 0,1,2,...\}$ is a family of semi-dynamical systems. Now for given $j \in \{1,2,...\}$ we get a fixed point for ψ_j.

Let j be fixed. We would like to find $x(j)$ which $\psi_j(x(j)) = x(j)$.

If $\psi_j(x(j)) = x(j)$ then $x(j+1) = x(j)$.

So that

$(x_1(j), x_2(j), ..., x_n(j)) = (x_1(j+1), x_2(j+1), ..., x_n(j+1))$.

$x_1(j+1) = x_1(j)$ implies $\sum_{k=1}^{n} \alpha_k(j)x_k(j) = x_1(j)$. If we put $\theta_k(j) = \alpha_k(j)$ $for\ all\ \ k = 2,...n$ and $\theta_1(j) = \alpha_1(j) - 1$ then

$$\sum_{k=1}^{n} \theta_k(j)x_k(j) = 0. \qquad (*)$$

For all k <j since $x_k(j+1) = x_k(j)$ and also $x_k(j+1) = \beta_{k-1}(j)x_{k-1}(j)$ we have

$$\begin{aligned} x_k(j) &= \beta_{k-1}(j)x_{k-1}(j) \\ &= \beta_{k-1}(j)\beta_{k-2}(j-1)x_{k-2}(j-1) \\ &= \beta_{k-1}(j)\beta_{k-2}(j-1)\beta_{k-3}(j-2)...\beta_1(j-(k-1)+1)x_1(j-(k-1)+1) \end{aligned}$$ $for\ all\ k$<j. (**)

For getting a fixed point we should have

$x_k(j) = x_k(j+1)$, $for\ \ all\ k$ >j .

So $x_k(j+1)=\beta_{k-1}(j)x_{k-1}(j)$ we have

$$x_k(j)=\beta_{k-1}(j)x_{k-1}(j)$$
$$=\beta_{k-1}(j)\beta_{k-2}(j-1)x_{k-2}(j-1)$$
$$=\beta_{k-1}(j)\beta_{k-2}(j-1)\beta_{k-3}(j-2)...\beta_{k-j-1}(0)x_{k-j-1}(0) \quad for\ all\ k>j. \quad (***)$$

At last we have $x_k(j)=x_k(j+1)$, $for\ k=j$ so

$$x_k(k)=\beta_{k-1}(k)x_{k-1}(k)$$
$$=\beta_{k-1}(k)\beta_{k-2}(k-1)x_{k-2}(k-1)$$
$$=\beta_{k-1}(k)\beta_{k-2}(k-1)\beta_{k-3}(k-2)...\beta_1(2)x_1(2) \quad . \quad (****)$$

So $p=x(j)=(x_1(j),x_2(j),...,x_n(j))$ is a fixed point if

$$\sum_{k=1}^{n}\theta_k(j)x_k(j)=0. \qquad (*)$$

$$x_k(j)=\beta_{k-1}(j)\beta_{k-2}(j-1)\beta_{k-3}(j-1)...\beta_1(j-(k-1)+1)x_1(j-(k-1)+1) for all\ k<j. \quad (**)$$

$$x_k(j)=\beta_{k-1}(j)\beta_{k-2}(j-1)\beta_{k-3}(j-2)...\beta_{k-j-1}(0)x_{k-j-1}(0) for all\ k>j. \quad (***)$$

$$x_k(k)=\beta_{k-1}(k)\beta_{k-2}(k-1)\beta_{k-3}(k-2)...\beta_1(2)x_1(2) \quad . \quad (****)$$

Since the equations (*), and one of the equations (**), or (***) or (****) have common solutions then the model has fixed point. Now we would like to find conditions for the hyperbolicity (Palis and de Melo, 1982) of a fixed point.

Let $\psi_{ji}:R^n\to R$ be the components of ψ_j, and p be a fixed point for it. Then

$$(d\psi_j)_p=\begin{bmatrix} \alpha_1(j) & \alpha_2(j) & . & . & . & \alpha_n(j) \\ \beta_1(j) & 0 & 0 & 0 & 0 & 0 \\ 0 & \beta_2(j) & 0 & . & . & 0 \\ 0 & .. & . & . & . & 0 \\ . & . & . & . & . & 0 \\ 0 & 0 & . & . & \beta_{n-1}(j) & 0 \end{bmatrix}.$$

Thus

$$\chi(c) := \det((d\psi_j)_p - cI) = \det\left(\begin{bmatrix} \alpha_1(j)-c & \alpha_2(j) & . & . & \alpha_{n-1}(j) & \alpha_n(j) \\ \beta_1(j) & -c & 0 & 0 & 0 & 0 \\ 0 & \beta_2(j) & -c & . & . & 0 \\ 0 & .. & . & . & . & 0 \\ . & . & . & . & -c & 0 \\ 0 & 0 & . & . & \beta_{n-1}(j) & -c \end{bmatrix}\right) =$$

$$(-1)^{n+1}\alpha_n(j)\begin{vmatrix} \beta_1(j) & -c & 0 & . & . & 0 \\ 0 & \beta_2(j) & -c & 0 & . & 0 \\ . & . & . & . & 0 & . \\ . & . & . & \beta_{n-3}(j) & -c & . \\ . & . & . & 0 & \beta_{n-2}(j) & -c \\ 0 & 0 & . & . & 0 & \beta_{n-1}(j) \end{vmatrix} +$$

$$(-c)(-1)^{n+n}\begin{vmatrix} \alpha_1(j)-c & \alpha_2(j) & . & . & . & \alpha_{n-1}(j) \\ \beta_1(j) & -c & 0 & 0 & . & 0 \\ . & . & . & . & . & . \\ . & . & . & . & 0 & . \\ . & . & . & \beta_{n-3}(j) & -c & 0 \\ 0 & 0 & . & . & \beta_{n-2}(j) & -c \end{vmatrix} =$$

$$(-1)^{n+1}\alpha_n(j)\beta_1(j)\beta_2(j)...\beta_{n-1}(j) + (-c)\alpha_{n-1}(j)(-1)^n\begin{vmatrix} \beta_1(j) & -c & 0 & . & . & 0 \\ 0 & \beta_2(j) & -c & 0 & . & 0 \\ . & . & . & . & . & . \\ . & . & . & . & -c & . \\ . & . & . & . & \beta_{n-3}(j) & -c \\ 0 & 0 & . & . & 0 & \beta_{n-2}(j) \end{vmatrix} +$$

$$(-c)^2(-1)^{n-1+n-1}\begin{vmatrix} \alpha_1(j)-c & \alpha_2(j) & . & . & . & \alpha_{n-2}(j) \\ \beta_1(j) & -c & 0 & 0 & . & 0 \\ . & . & . & . & . & . \\ . & . & . & . & 0 & . \\ . & . & . & . & . & 0 \\ 0 & 0 & . & . & \beta_{n-3}(j) & -c \end{vmatrix} = ... =$$

$$(-1)^{n+1}\alpha_n(j)\beta_1(j)\beta_2(j)...\beta_{n-1}(j) +$$
$$(-c)(-1)^n\alpha_{n-1}(j)\beta_1(j)\beta_2(j)...\beta_{n-2}(j) +$$
$$(-c)^2(-1)^{n-1}\alpha_{n-2}(j)\beta_1(j)\beta_2(j)...\beta_{n-3}(j) +$$
$$(-c)^3(-1)^{n-2}\alpha_{n-3}(j)\beta_1(j)\beta_2(j)...\beta_{n-4}(j) + ... +$$
$$(-c)^{n-2}(-1)^3\alpha_2(j)\beta_1(j) + (-c)^{n-1}(-1)^2(\alpha_1(j)-c) =$$

Thus

$$\chi(c) = (-1)^{n+1}\{\alpha_n(j)\beta_1(j)\beta_2(j)...\beta_{n-1}(j) +$$
$$c\alpha_{n-1}(j)\beta_1(j)\beta_2(j)...\beta_{n-2}(j) +$$
$$c^2\alpha_{n-2}(j)\beta_1(j)\beta_2(j)...\beta_{n-3}(j) +$$
$$c^3\alpha_{n-3}(j)\beta_1(j)\beta_2(j)...\beta_{n-4}(j) + ... +$$
$$c^{n-2}\alpha_2(j)\beta_1(j) + c^{n-1}(\alpha_1(j) - c)\}.$$

So the fixed point $p = x(j)$ is a hyperbolic fixed point if $\chi(-1) \neq 0$ and $\chi(1) \neq 0$ .this model also contains the non hyperbolic fixed points. For example if we put $n = 4$, and $j = 2$, and consider a model with $\alpha_1(2) = 6,\ \alpha_2(2)\beta_1(2) = -2,\ \alpha_3(2)\beta_1(2)\beta_2(2) = 0,$ and $\alpha_4(2)\beta_1(2)\beta_2(2)\beta_3(2) = -3,$ then this model satisfies the equations (*) and

(****). So we have a fixed point for ψ_2. This fixed point is not a hyperbolic fixed point because $\chi(c) = c^4 - 6c^3 + 2c^2 + 3,\ \chi(1) = 0$.

Conclusion

In the previous section we stated the conditions which can lead us to find conditions to fix the number of a special age group.
It can be very helpful in the developing programs of the society, which keeping the number of a particular age group of human being fixed, and this model has the stability for such programming.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LIII, 1, 2008, p. 51-58

SPECTROFLUOROMETRIC ANALYSIS OF CHICKEN IGY STABILITY AFTER UREA TREATMENT

FLORINA OLARU[1], ALINA SESARMAN[1], HORIA BANCIU[1], MANUELA BANCIU[1], IOAN PETRESCU[1]

SUMMARY. Spectrofluorimetric measurements are useful tools to investigate structural changes in proteins. More insights into the protein folding pathways are essential for the general understanding of the antibody molecule structure. Moreover, elucidation of the folding mechanisms plays a crucial role in illustrating the final configurations of multidomain oligomeric proteins. The fact that chicken IgY antibodies might represent exquisite tools in diagnostics, medical application or biotechnology prompted us to investigate the effect of chemical conditions-urea treatment on IgY stability, by monitoring changes in fluorescence emission spectra. Our data revealed that IgY stability is not affected after 3-5 hours incubation with 0-9M urea, denaturation occuring only after 21h incubation with 5,5-9.5M urea. The low fluorescence intensity of ANS bound to IgY, appears to indicate no conformational changes after urea treatment, probably due to the lack of hydrophobic sites on IgY immunoglobulin. Increase stability in different urea conditions would suggest a possible therapeutic role of IgY antibodies.

KEYWORDS: IgY immunoglobulin, denaturation, renaturation, urea.

Introduction

Among all avian immunoglobulins (Ig), chicken IgY immunoglobulins are most frequently studied and best characterized. IgY antibodies represent the counterpart of mammalian IgG, although, structurally and functionally, they are closely related to IgE (Warr et al., 1995). The structure differences between chicken IgY and mammalian IgG, are reflected in their biological function.

In terms of immunological properties, chicken IgY antibodies have several advantages over mammalian IgG: 1) the lack of reactivity with human (Lindahl et al., 1992) or bacterial Fc receptors (Godfrey et al., 1992, Hoffman et al., 1996); 2) the inability to activate mammalian complement cascade (Gigli and Austen, 1971, Larsson et al., 1992, Vikinge et al., 1998); 3) the lack of interaction with rheumatoid factors (RF) and human anti-mouse IgG antibodies (HAMA) (Larsson et al., 1991, Schade and Hlinak, 1996); 4) evolutionary difference, which confers

[1] *Faculty of Biology and Geology, Babeş-Bolyai University, 400006 Cluj-Napoca, Romania. E-mail: alina.sesarman@mail.ubbcluj.ro*

IgY an increased ability to recognized more epitope on a mammalian protein than the corresponding mammalian IgG (Gassmann et al., 1990); 5) increased resistance under various physico-chemical conditions. Due to this differences, IgY antibodies represent excellent tools with application in various areas of research: diagnostics, medical application or biotechnology (Narat, 2003).

In the present paper, we examine the effect of different urea concentrations on the stability of IgY antibodies, by measuring the changes in either the intrinsic fluorescence of the tryptophan residues of IgY, or the extrinsic fluorescence after administration 1-anilino-8-naphthalene sulfonate (ANS). Changes in tryptophan fluorescence wavelenghts is a widely used tool to observe changes such as folding/ unfolding dynamics in proteins (Ervin et al., 2000). ANS has been used extensively as a fluorescent probe to monitor hydrophobic sites of proteins, after denaturation (Strayer, 1965).

The molecular mechanisms of the denaturing action by urea is not entirely elucidated, albeit, it is believed that the denaturant action occurs due to preferentially interaction with the backbone CO-NH groups and other polar groups in the side-chains by forming multiple H-bonds, leading to the *salting in* of such groups in the aqueous solution (Nandi and Robinson, 1984, Roseman and Jencks, 1975) the addition of urea to water causes an increase of the surface tension , leading to the *salting out* of nonpolar groups from the aqueous phase (Breslow and Guo, 1990). Increase stability in different urea conditions would suggest a possible therapeutic role of IgY antibodies.

Materials and methods

*Denaturation studies of IgY antibodies***.** IgY antibodies were purified from chicken egg yolk (27.7μM) and subsequently incubated in 0-9 M urea at 25ºC, for 3, 5 and 21 hours. The state of protein denaturation was monitored by measuring the changes in both intrinsic and extrinsic fluorescence intensities (Jasco FP-750 spectrofluorometer).

In the case of intrinsic tryptophan fluorescence recording, the urea denaturation mixture was diluted ten times with 10 mM sodium phosphate saline buffer (PBS), pH-7,2. The excitation wavelength was set at 295 nm and the fluorescence emission spectrum was recorded in the range 300-420 nm.

The extrinsic fluorescence was obtained by using 55.5 μM ANS (1-anilino-8-naphthalene sulfonate) that binds to cationic groups of proteins (Matulis et al., 1999). After denaturation, each mixture was diluted five times with 10 mM PBS, pH 7.2. The excitation wavelength was set at 350 nm and the fluorescence emission spectra were recorded in the range 420-600 nm.

Renaturation studies of IgY antibodies. For the renaturation studies, the urea denaturation mixtures were diluted in 10 mM PBS (pH - 7.2) solution as follows: ten times, in the case of measuring of intrinsic fluorescence intensity and five times with the same buffer in the case of measuring of extrinsic fluorescence

intensity. The time of incubation was 3, 5 and 21 hours at 25ºC. Fluorescence excitation and emission wavelengths are the same with those used for denaturation studies (Covaciu *et al.*, 2004a; Covaciu *et al.*, 2004b).

Results and discussion

Proteins are synthesized as a linear chain of amino acids, but a functionally protein has a folded (native) conformation. At physiological value of pH, the folded and unfolded states of a protein are in equilibrium. The stability of the native state in aqueous solutions is influenced by: the amino acid sequence of the protein, the pH conditions, the temperature and the concentration of salts and ligands. Immunoglobulins, show a strong structure-function relation in the different domains, which makes these molecules excellent systems for various tests. Herein, we evaluated the effect of increasing urea concentrations, on IgY stability, based on changes in the intrinsic or extrinsic fluorescence intensitiy, due to the presence of tryptophan and ANS bound to IgY.

The intrinsic tryptophan fluorescence emission spectra of IgY in urea conditions. The state of denaturation-renaturation of IgY was studied by measuring the changes of intrinsic fluorescence intensity at 341 nm and of extrinsic fluorescence intensity at 474 nm. For the intrinsic and extrinsic fluorescence studies, the concentrations of IgY were 2.77 μM and 5.55 μM respectively. After 3 hours of incubation with different urea solutions (0-9.5M), the intrinsic tryptophan fluorescence emission spectra of IgY were similar. The maximum fluorescence intensity at 341 nm has the same values (369% to 405%) in the range of 0-9.5M urea (Fig. 1a). After 5 hours of incubation the intrinsic fluorescence intensities of IgY were constant (about 430%) up to 5.5 M urea. The fluorescence intensity increases slightly from 437% to 585% with 9.5 M urea (Fig. 1b). In the range of 0-5.5 M urea, the fluorescence intensities are similar, with values between 369%-380%. The same situation was observed after 21 hours of incubation of IgY with urea (Fig. 1c). No significantly difference could be observed when comparing the fluorescence intensity values of IgY after 3 hours of incubation with 9.5M urea (405%) and 0M urea (369%). After 5 and 21 hours of incubation of IgY with 9.5M urea, the fluorescence intensity values (585% and 583% respectively) were similar (Fig. 1d). The values of intrinsic fluorescence intensity of IgY in 0-9.5M urea solutions, after 3 (Fig. 1e) and 21 (Fig. 1f) hours of incubation with renaturation buffer, were similar with those from denaturation.

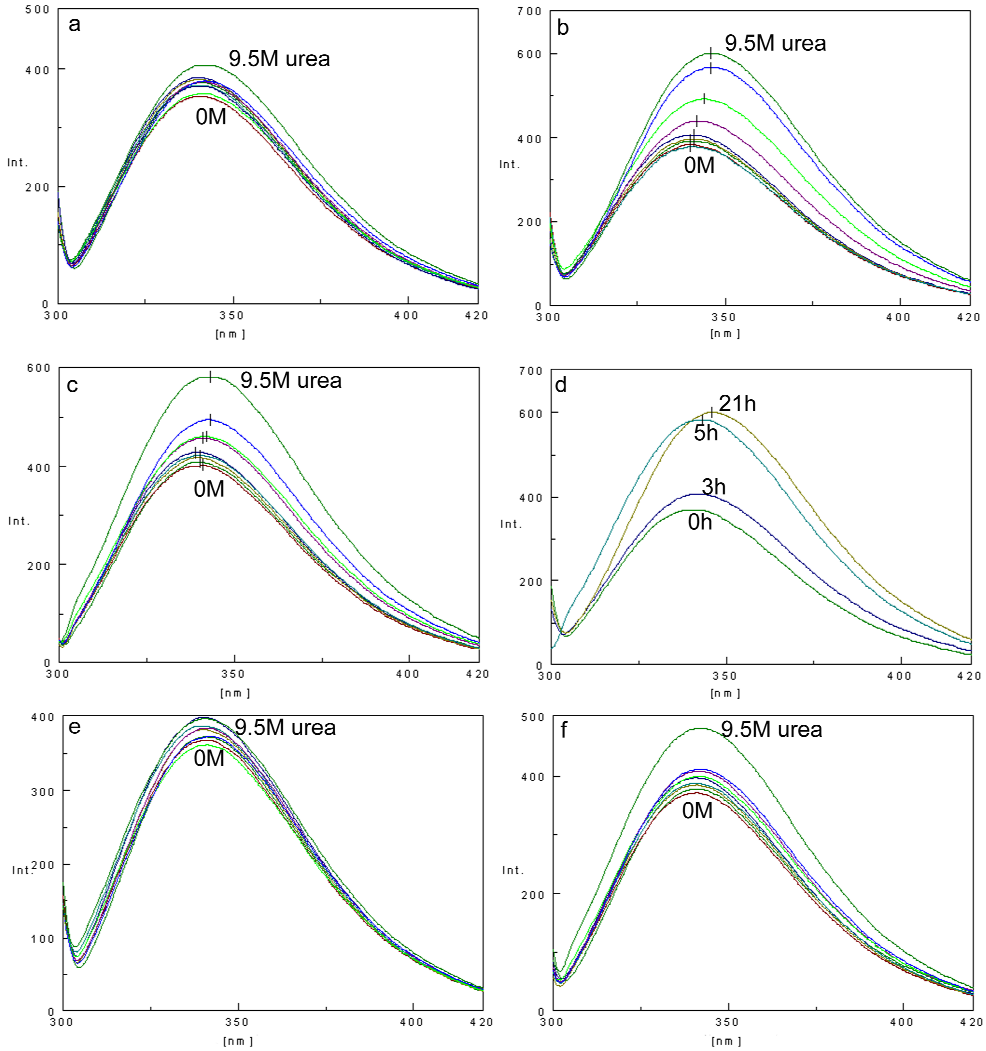

Fig. 1. The intrinsic tryptophan fluorescence emission spectra of IgY in urea conditions. Intrinsic fluorescence emission spectra of IgY after 3 (a), 5 (b) and 21 (c) hours of incubation with 0-9,5M urea; (d) Comparison of the fluorescence emission spectra of IgY after 3, 5 and 21 hours. Intrinsic fluorescence emission spectra of IgY after 3 (e) and 21 (f) hours of incubation with renaturation buffer.

The fluorescence emission spectra of ANS bound to IgY in urea conditions. The IgY ANS-binding affinity was weak. The maximum extrinsic fluorescence intensities at the peak wavelength 474 nm were 36.96% for the IgY (5.55μM). The values of extrinsic fluorescence intensity were constant in the range of 0-9.5 M urea. After 3 or 5 hours of incubation in denaturation (Fig. 2a) or renaturation (Fig. 2b) conditions, significantly changes in the extrinsic fluorescence emission spectra of ANS bound to IgY, could not be detected.

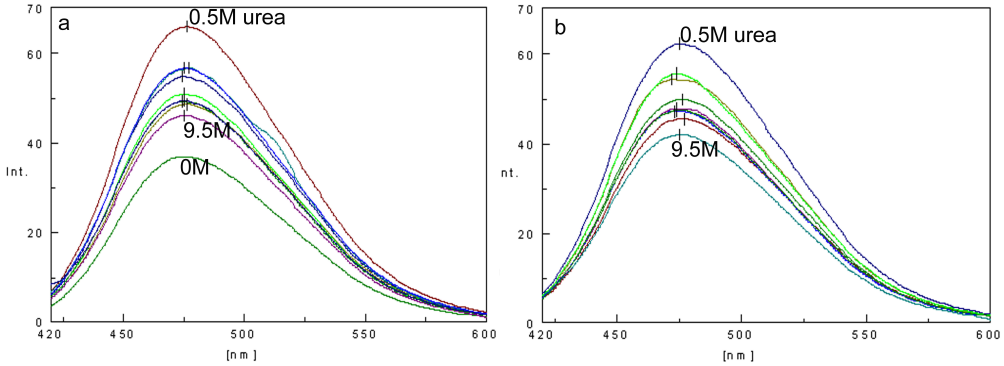

Fig. 2. The fluorescence emission spectra of ANS bound to the IgY in urea conditions. (a) Fluorescence emission spectra of ANS bound to IgY after 3 hours of incubation with 0-9,5M urea; (b) Fluorescence emission spectra of ANS bound to IgY after 3 hours of renaturation.

This low capacity of ANS binding is probably due to the low number of cationic amino acids from the IgY composition.

Comparison of urea denaturation–renaturation profiles of IgY. After 3 hours of incubation, the fluorescence intensities at all urea concentrations were similar (about 370%). The same results were obtained after incubation in the renaturation buffer. These results could suggest that IgY is resistant to denaturation in the range of 0-9.5 M urea after 3 hours of incubation. (Fig. 3a). After 5 hours of incubation, the values of fluorescence intensity at all urea concentrations increase in the range of 5.5-9.5M urea (about 437%-585%), suggesting a possible denaturation in this domain (Fig. 3b). A slightly denaturation have been seen after 21 hours in the range of 0.5-5.5 M urea. After incubation in the renaturation buffer, the values of the intrinsic fluorescence intensity were similar with the one obtained at denaturation (370%-380%), suggesting that IgY is partially renaturated in the range of 0.5-5.5 M urea after 21 hours of incubation. The values of fluorescence intensity increased in the range of 5.5-9.5 M urea, suggesting an irreversible denaturation (Fig. 3c). Fig. 3d shows the comparative denaturated profile of IgY at different time of measuring. In the case of the ANS binding to IgY, the values of extrinsic fluorescence intensity were constant, increasing slightly at 1.5 M urea (65.8%)

suggesting a reversible denaturation in the range of 0.5-3.5 M urea. After incubation in the renaturation buffer, the fluorescence intensities at all urea concentrations were similar with those from denaturation step (Fig. 3e). The fluorescence intensities were unchanged after 5 hours of incubation (Fig. 3f)**.** These results suggest a low number of cationic amino acids from IgY structure.

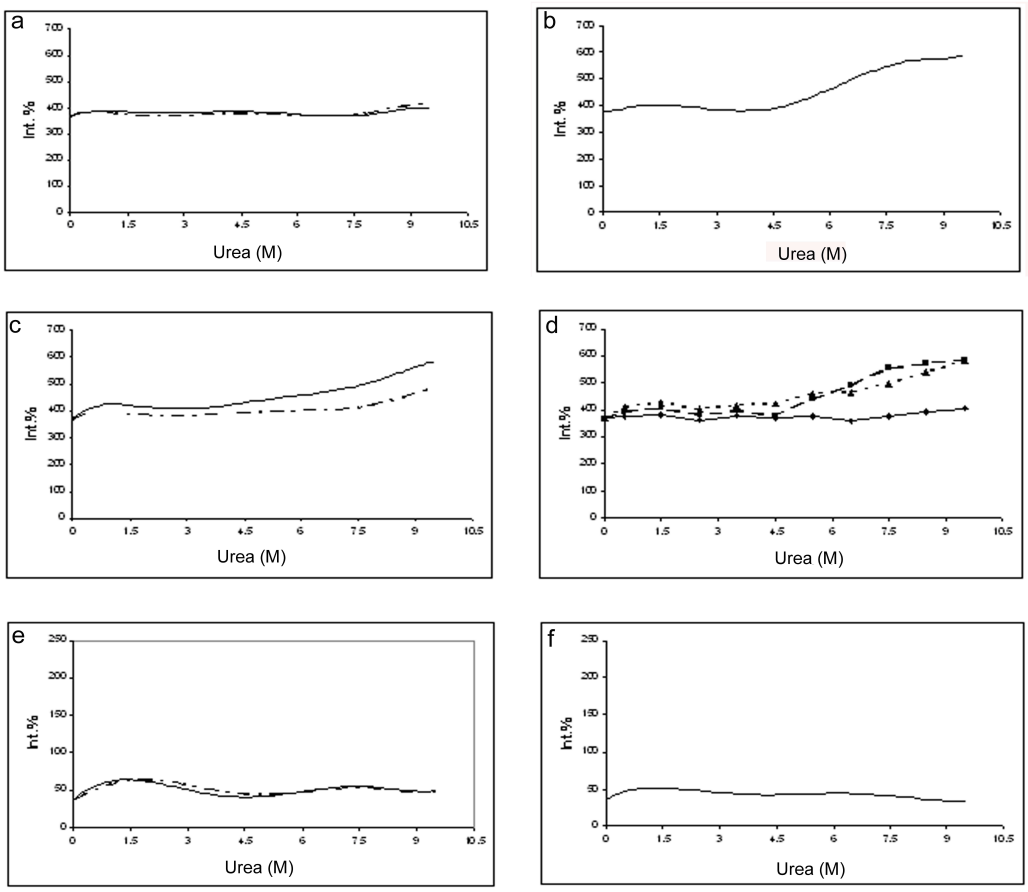

Fig. 3. Comparison of urea denaturation–renaturation profiles of IgY. Denaturation-renaturation of IgY after 3 (a), 5 (b) and 21 (c) hours (intrinsic fluorescence). (d) Comparison of denaturation IgY profiles after 3, 5 and 21 hours (solid line–3 hours; dashed line–5 and 21 hours). Denaturation-renaturation of IgY after 3 (e) and 5 (f) hours (extrinsic fluorescence); solid line–denaturation; dashed line–renaturation; Int.(%)-fluorescence intensity

Conclusions

Our experimental data indicate that IgY stability is not significantly affected after 0- 9.5M urea treatment at room temperature. A slightly increase of fluorescence intensity, in the range of 5.5-9.5M urea, after 21 hours incubation, suggests a possible irreversible denaturation of IgY. The low values of fluorescence intensity of ANS bound to IgY in urea conditions, indicates no conformational changes, probably due to lack of cationic sites on IgY. A further investigation of the stability of IgY antibodies against urea, using different experimental techniques should be useful to confirm this evidences.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LIII, 1, 2008, p. 59-69

THE MOLECULAR DIVERSITY OF CYANOBACTERIAL MATS ASSOCIATED WITH THERMAL SPRINGS IN NORTH-WESTERN ROMANIA

BOGDAN DRUGĂ[1,2], IULIA SOFRONIE[1], IOANA VĂSAR[1], CRISTIAN COMAN[1,2], ADRIANA BICA[1,2], ANA NICOARĂ[2], NICOLAE DRAGOŞ[1,2]

SUMMARY. In this study, the diversity of the cyanobacterial communities associated with the thermo-mineral springs was analyzed. We used a molecular technique based on the generation of different electrophoretic patterns, subsequent to a DNA digestion with restriction enzymes. The target DNA fragment which was amplified through PCR was the 16S rRNA / ITS gene, a well known molecular marker used in the phylogenetic studies of the prokaryotes. The 16S rRNA / ITS gene wes cloned in *E. coli* cells, using a plasmidic vector. Eventually, we have obtained 24 to 45 different clones for the 4 cyanobacterial communities. The target DNA fragments were re-amplified through PCR using the plasmids as template DNA, and then the 16S rRNA / ITS gene copies were digested with several restriction enzymes. Of all, only the TaqI restriction enzyme has generated unambiguous electrophoretic bands, and specific patterns. The distances the digested DNA fragments have migrated into the electrophoretic gel were measured. The number of distinct electrophoretic patterns is considered the same with the number of different cyanobacterial species from a related mat.

KEYWORDS: 16S rRNA, ITS, DNA cloning, restriction map

Introduction

Cyanobacteria are photosynthetic prokaryotes that occur in widely different habitats ranging from fresh, brackish and marine waters to hot springs, damp or Antarctic soils, deserts or hyper saline lakes (Van den Hoek *et al.,* 1997, Rasmunsen and Svenning, 1998, Mazel *et al.* , 1990). Developments in gene-based identification methods and environmental-analysis techniques have significantly enhanced the ability to describe the ecology of bacterial populations in situ (Ferris *et al.,* 2003, Taton *et. al*., 2003). Our research has focused on the molecular diversity of cyanobacteria in hot spring mats, where cellular morphologies are of limited use in distinguishing populations. We analyzed the 16S rRNA gene, well

[1] *Faculty of Biology and Geology, Babeş-Bolyai University, 5-7 Clinicilor Street, 400006/Cluj-Napoca, Romania*
[2] *Institute of Biological Research, 48 Republicii Street, 400015/Cluj-Napoca, Romania*

known as suitable molecular marker in the phylogeny of the prokaryotes, due to its enhanced conservation level in time (Otsuka *et al*., 1998, Janse *et al*., 2004, Lee and Kyung, 2001, Katano *et al*., 2001).

Microscopy has always been essential for studying the morphology of microbial mats, and light microscopy has been the mainstay of such research (de los Rios, 2003). Nevertheless, the microscopy may be unable to discriminate between different species from field samples, as long as these samples usually contain one or two dominant species, and the remaining ones could pass unobserved (Myers *et al*., 2007). This is the reason for which we employed this molecular method for the study of the cyanobacterial communities from four thermal springs in the west of Romania.

Materials and methods

Sampling sites and morphological studies. The cyanobacterial mats that were studied in this work were sampled from four thermal springs, in Satu Mare and Bihor counties (Table 1). Part of the biological material was frozen in liquid nitrogen, to be preserved for the DNA extraction. Furthermore, some samples were kept in liquid medium in order to be analyzed with the optical microscope.

Table 1.

The four locations where the cyanobacterial samples were collected

Sampling place	Lat / Long	Temperature (^{0}C)	pH
Beltiug	47^0 53’ 22’’ / 22^0 85’ 24’’	59	7. 95
Ady-Endre	Not determined	48. 3	8. 38
Săcuieni	47^0 21’ 31’’ / 22^0 05’ 64’’	77	7. 64
Ciocaia	47^0 19’ 97’’ / 22^0 03’ 09’’	65	7. 74

Part of the samples brought in the laboratory was passed in GZ liquid medium, and after their growth, they were used for an additional DNA extraction.

The cell morphology was analyzed with a Nikon Eclipse TE2000-U inverted microscope and photographed with a Nikon D200 digital camera. The best results were achieved with the 100X immersion objective (Fig. 1).

Fig. 1. Optical microscope images with cyanobacterial mats from Beltiug and Săcuieni

DNA extraction. Cyanobacterial DNA was extracted using a cyanobacterial-specific protocol, based on phenol, chloroform and isoamilic alcohol purification. In addition to this protocol, we have first treated the biological samples with aluminium sulfate, in order to remove the humic acids. These compounds are wide-spread in any kind of soil and they are well known for their capacity to inhibit the chemical reactions subsequent to DNA extraction, including the PCR (Dong et al., 2005, Moreira, 1998, Bürgmann et al., 2000, Bachoon et al., 2001). After the removal of the humic acids, we followed the specific steps of the protocol. Thus, the samples were washed with certain buffers, and then were treated with Sarcosyl, which is a detergent whose function is to remove the peptidoglycan layer from the cyanobacterial surface. The subsequent steps include the sample treatment with Sodium Dodecyl Sulfate and K Proteinase, in order to denaturate any proteic structure that may affect the DNA quality. DNA isolation was achieved by phenol addition in the same quantity with that already existent in the tubes. The phenol and chloroform function in this method is to denature the proteins. DNA precipitation was accomplished with the addition of NaCl and isopropyl alcohol. After gently stirring up the samples, DNA could be observed as a transparent aggregate. The obtained DNA was preserved in refrigerator, at -20^{o}C (Ausubel et al., 2000).

DNA was also extracted with two commercial kits, provided by Promega and Fermentas. In both cases, the biological samples were first treated with aluminium sulfate, for the humic acids removal. In the subsequent steps, we used the DNA extracted with all these methods, in function of the DNA quality obtained from each sample.

PCR and electrophoresis of the products. The amplification reactions were performed employing the PCR (Polymerase Chain Reaction) with a "Tgradient Thermocycler", produced by Biometra. For the amplification of the 16S rRNA and ITS region (Fig. 2) -about 2000 bp- we used primers 27F and ITER (Table 2). For a final volume of 50 µl reaction mixture we have used: 32. 5 l ultra pure water, 10 µl 5x Flexy Green Buffer, 3 µl $MgCl_2$ (25 mM), 1 µl dNTP, 1 µl ITER, 1 µl 27F, 1 µl DNA and 0. 5 µl Go Taq Polymerase. The temperature profile was: one cycle of 94^{o}C, 5 min; 30 cycles of 94C, 1 min; 65^{o}C, 75 seconds; 72^{o}C, 75 seconds; one cycle of 72^{o}C, 15 min.

The PCR products were purified using electrophoresis on 1. 0% agarose gel and Promega Purification Kit.

Table 2.

The primers used in DNA amplification

Primer	Formula
27F	5' - AGA GTT TGA TCM TGG CTC AG - 3'
ITER	5' - CTC TGT GTG CCT AGG TAT CC - 3'

Cloning the 16S rRNA gene and the ITS. The PCR products were cloned in the pGEM-T Vector (Promega) using manufacturer's instructions. The vector contains a multiple cloning frame which lies within the α-peptide coding. The ligation reaction contained: 25 ng PCR product, 3 units T4 DNA ligase, 2x Rapid Ligation Buffer T4 DNA ligase, deionized water to a final volume of 10 µl. The reactions took place one hour at room temperature. After electroporation the *E. coli* DH5α cells became competent, and they were incubated together with the plasmid 1 hour at 37^{o}C.

From this mix 120 µl were pipetted on LB/ampicillin/IPTG/X-Gal plates (double selection, with ampicillin on one hand and X-Gal on the other hand) which were incubated overnight at 37^{o}C. A various number of white and blue colonies per plate are usually observed. White colonies generally contain the insert.

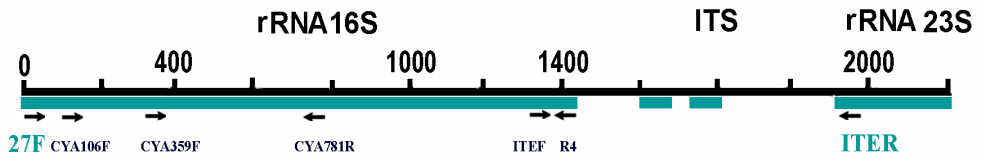

Fig. 2. Partial rrn operon and the relative positions of primers during annealing. With green are marked 16S and 23S rRNA. Between them lies ITS.

Each one of these white colonies was passed onto a tube containing liquid LB medium, ampicillin added. After cloning the interest DNA fragment, we have performed the plasmid isolation with commercial kits provided by Fermentas and Promega. For each studied cyanobacterial mat, we have performed over one hundred cloning procedures, in order to achieve as many different clones as possible.

All the obtained clones were subjected to PCR amplification for the 16S rRNA / ITS gene. Eventually, all amplicons were digested with TaqI, HaeIII, RsaI and AluI restriction enzymes, all provided by Fermentas. The reaction mixture contains 17 µl nuclease-free water, 2 µl fast digest 10x buffer, 10 µl DNA amplicon and 1 µl restriction enzyme (1 FDU/ µl). The reactions took place at 37^0C, except the case of TaqI, where the proper temperature for the suitable function of the enzyme is 65^0C. The digested DNA samples were electrophoretically migrated in 1% agarose gel, at a 5 V/cm voltage.

We used the amplicons digestion in order to obtain a method for a fast discrimination between the DNA fragments resulted from different cyanobacterial species.

Results and discussion

After the PCR amplification with the CYA 27F and ITER primers we obtained a 1800-2000 nucleotide fragment, consisting in the 16S rRNA gene and the internal transcribed spacer (ITS) between this gene and the 23S rRNA gene. This fragment was cloned, and the recombinant plasmids were then extracted (Fig. 3). Eventually, we obtained 24 clones from Ciocaia sample, 28 from Ady-Endre, 29 from Săcuieni and 45 from Beltiug.

All the resulted PCR amplicons were eventually digested with four restriction enzymes: TaqI, HaeIII, RsaI and AluI. Of all, the last three have generated many ambiguous electrophoretic bands, as well as diffuse smears between them (data not shown). On the other hand, the TaqI restriction enzyme has generated unambiguous electrophoretic bands. The number of distinct electrophoretic patterns is considered the same with the number of different cyanobacterial species from a related mat.

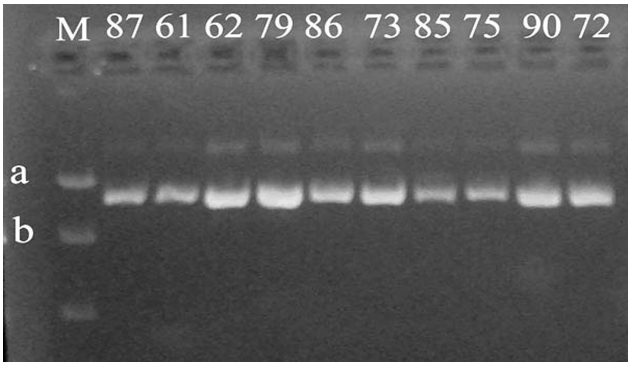

Fig. 3. Recombinant plasmids extracted from *E. coli* cells, after cloning the 16S rRNA / ITS gene from the Beltiug DNA sample. M = control; a = 5000 bp; b = 2000 bp. The pGEM-T plasmid is 3003 bp long, and the DNA insert is 1800-2000 bp long.

The resulted recombinant plasmids were subjected to PCR amplification with the 27F and ITER primers, in order to achieve a large numbers of 16S rRNA amplicons (Fig. 4).

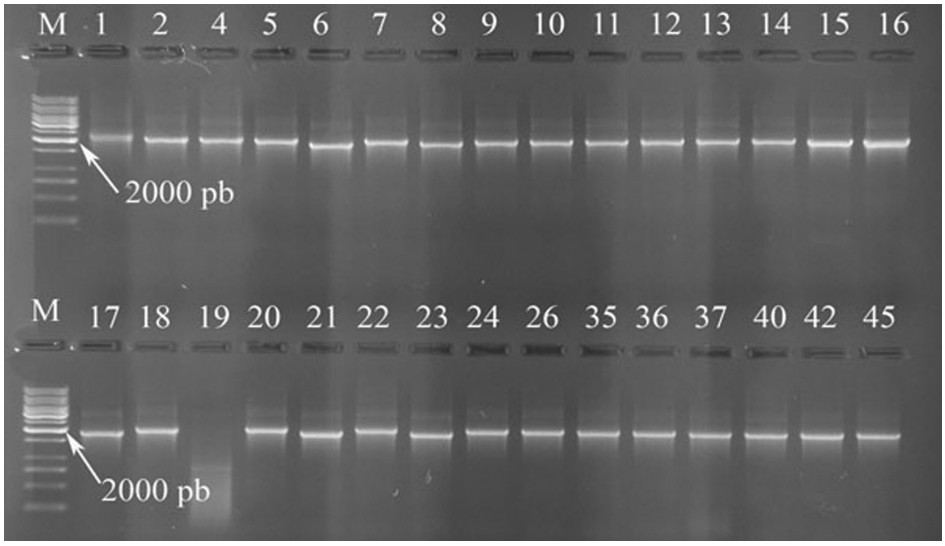

Fig. 4. Electrophoregram with the 16S rRNA / ITS amplicons obtained from the Săcuieni DNA samples subsequent to the PCR amplification on recombinant plasmids

In the case of the Beltiug cyanobacterial mat, we obtained 45 different clones of the 16S rRNA/ ITS gene. As presented before, we did not obtain specific electrophoretic patterns with the restriction enzymes, except the TaqI. This enzyme derives from the bacteria *Thermus aquaticus*, and its proper temperature for use is 65^0C. Between the 45 clones, we have concluded that there are 7 different electrophoretic patterns (Fig. 5). The distance between the bands was measured with the LUCIA Net software, in correlation with the control bands. The DNA fragments corresponding to the electrophoretic bands were ranging from 100 to 827 bp long.

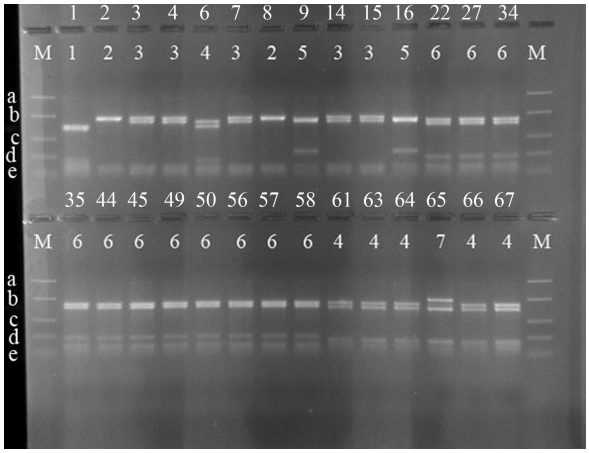

Fig. 5 The electrophoretic pattern of 28 of the 45 16S rRNA / ITS amplicons from Beltiug, digested with the TaqI restriction enzyme. M = control; a = 1500 bp; b = 850 bp, c = 400 bp; d = 200 bp; e = 50 bp. The upper numbers represent the number of the clones, while the bottom numbers represent the 7 distinct patterns

With the sample DNA from the Săcuieni cyanobacterial mat, we have obtained 29 different clones of the 16S rRNA/ITS gene. The digestion with the TaqI restriction enzyme has generated 6 different electrophoretic patterns, probably corresponding to 6 different OTU (Operational Taxonomic Unit), or species (Fig. 6). The DNA fragments corresponding to these bands ranged between 170 and 975 bp.

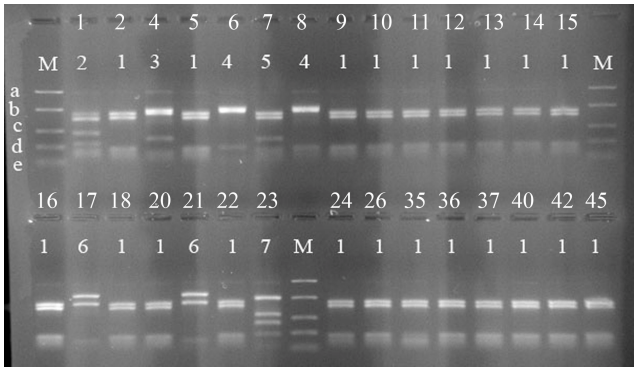

Fig. 6 The electrophoretic pattern of the 16S rRNA / ITS amplicons from Săcuieni, digested with the TaqI restriction enzyme. M = control; a = 1500 bp; b = 850 bp, c = 400 bp; d = 200 bp; e = 50 bp. The upper numbers represent the number of the clones, while the bottom numbers represent the 7 distinct patterns

In the case of the Ady-Endre cyanobacterial mat, we obtained 28 different clones of the 16S rRNA/ITS gene. After the digestion with the TaqI restriction enzyme, we obtained 4 different electrophoretic patterns. The bands have corresponded to various DNA fragments with the length between 104 and 738 bp (Fig. 7).

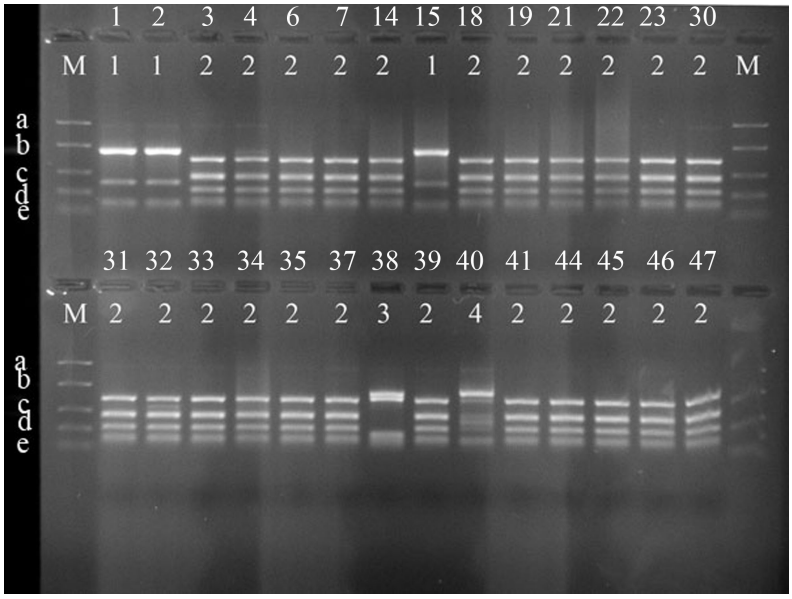

Fig. 7 The electrophoretic pattern of the 16S rRNA / ITS amplicons from Ady-Endre, digested with the TaqI restriction enzyme. M = control; a = 1500 bp; b = 850 bp, c = 400 bp; d = 200 bp; e = 50 bp. The upper numbers represent the number of the clones, while the bottom numbers represent the 4 distinct patterns

From the DNA belonging to the Ciocaia mat, we have obtained 24 different 16S rRNA / ITS gene clones. Their digestion with the TaqI restriction enzyme has generated 5 specific electrophoretic patterns (Fig. 8).

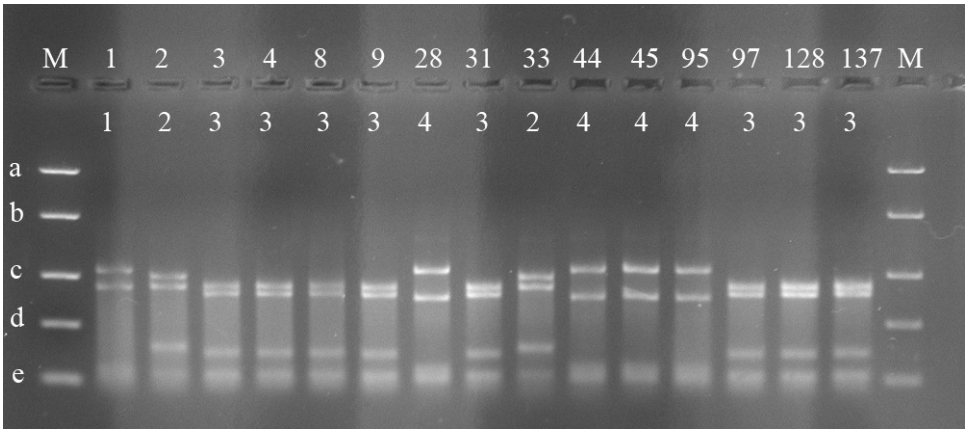

Fig. 5 The electrophoretic pattern of 15 of the 24 16S rRNA / ITS amplicons from Ciocaia, digested with the TaqI restriction enzyme. M = control; a = 5000 bp; b = 2000 bp, c = 850 bp; d = 400 bp; e = 100 bp. The upper numbers represent the number of the clones, while the bottom numbers represent the 4 distinct electrophoretic patterns

In this study method we have succeeded to analyze the diversity encountered in the cyanobacterial mats associated with the hot springs. This is a less expensive way to analyze the microbial communities, and for this study to be fulfilled, we will perform the sequencing of the different clones, in order to determine all the different species which compose these mats. Another proper method for the study of the bacterial communities has proved to be the molecular analysis by DGGE (Denaturing Gradient Gel Electrophoresis)**.** Although this method is relatively expensive, it is well known that it is very accurate, as long as it allows the discrimination between DNA fragments which differ by a single nucleotide. For the future studies, we consider the DGGE analysis as an alternative to certify the results gained during this work.

Conclusions

Our work has confirmed that in the case on the cyanobacterial mats sampled from different soils, the treatment with aluminium sulfate largely removes the humic acids which may affect the chemical reactions of DNA in the subsequent steps.

Of all the employed DNA restriction enzymes, the TaqI has proved to be the proper one in order to obtain unambiguous electrophoretic patterns, with clear DNA bands. The other restriction enzymes used in this study have generated unspecific patterns, with diffuse smears between the DNA bands. This result recommends the TaqI restriction enzyme for further restriction analyses.

The study of the restriction patterns of the 16S rRNA / ITS clones has proved to be a suitable method for the analysis of the cyanobacterial mats, as long as it gave us the number of different species which characterize the studied communities.

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A MOLECULAR APPROACH TO INVESTIGATION OF CYANOBACTERIAL DIVERSITY FROM MARGHITA AND ROŞIORI THERMOMINERAL DRILLINGS (BIHOR COUNTY, ROMANIA)

CRISTIAN COMAN[1,2], BOGDAN DRUGĂ[1,2], ADRIANA BICA[1,2], ANA NICOARĂ[1], NICOLAE DRAGOŞ[1,2]

SUMMARY. Understanding the relationships among species in different microbial communities, in time, has proved to be quite a challenge. The fact that the majority of species are not culturable in laboratory conditions and determination based on morphological characteristics is sometimes very difficult, emphasized the need of a new approach in this type of studies. Based on a solid biomarker – the gene for 16S rRNA and 16S-23S ITS region – two specific cyanobacterial mats associated to Marghita and Roşiori thermomineral drillings were investigated using molecular techniques. The 16S rRNA-ITS fragments from the entire community genomic DNA were amplified through PCR using specific cyanobacterial primers, the fragments were cloned and a restriction analysis was performed. The restriction maps obtained revealed the presence of 13 groups 16S RNA-ITS profiles for Marghita and 2 groups 16S RNA-ITS profiles for Roşiori, suggesting the presence of a similar number of distinct operational taxonomic units (OTU's). Distinct fragments were sequenced and partial 16S rRNA sequences obtained were compared to the ones stored in public databases (NCBI GenBank) using *blastn* algorithm for specie identification. At the same time a phylogenetic analysis was conducted.

KEYWORDS: Cyanobacteria, thermomineral drillings, 16S rRNA, diversity phylogeny

Introduction

Defining the diversity and structure of natural microbial communities through the quantification of their constituent populations has been a long-standing challenge in microbial ecology. Selective enrichment cultivation as an approach for the description of naturally occurring microbial communities has severe limitations (Poindexter *et al*., 1986, Ward *et al.*, 1992) because the majority of bacteria in nature cannot be cultivated using traditional techniques (Brock *et al., 1987*, Jannasch *et al.*, 1959).

[1] *Institute of Biological Research, 48 Republicii Street, 400015/Cluj-Napoca, Romania*
[2]*Faculty of Biology and Geology, Babeş-Bolyai University, 5-7 Clinicilor Street, 400006/Cluj-Napoca, Romania*

The study of diversity in certain microbial communities through classic methods, based on phenotypic identification of taxa, was more and more recognized that provides inconclusive information. This is because these methods cannot determine genetic variability and, in many cases, they induce misunderstandings because phenotype diversity is not always correlated with genetic diversity.

An alternative approach to understanding the composition of natural communities is the one that uses molecular biology techniques and provides a culture-independent analysis of microorganisms.

Cyanobacterial mats associated to thermomineral drillings are an excellent model for studying molecular diversity and colonizing potential of cyanobacteria because they have precise spatial delimitation and homogenous conditions enforced by constant temperature and water chemistry. They also represent an important source of genes that encode proteins resistant to high temperature.

In this study our objective is to investigate diversity and community structure of two cyanobacterial mats associated with Marghita and Roşiori thermomineral drillings by a molecular approach. The question is: can molecular techniques be used independently of morphology to characterize microbial communities? To answer this question we selected a marker widely used for this type of studies – 16S rDNA and 16S-23S rDNA Internal Transcribed Spacer (ITS) – and we applied several molecular manipulations using this marker. The 16S rRNA gene contains information that makes it a good biomarker of microorganisms due to the fact that this gene contains both highly conserved regions found in all living organisms and diagnostic variable regions that are unique to particular organisms or closely related groups of organisms (Moyer *et al*., 1995). Besides the 16S rRNA gene we also used the 16S-23S Internal Transcribed Spacer (ITS) which, based on its high variability, could be used for strain differentiation. For this purpose, we performed restriction map analysis, cluster analysis, sequencing and phylogeny reconstruction. The restriction maps and sequences were obtained from different clones derived from a library of cyanobacterial 16S rRNA gene and ITS. Based on the restriction map, we sequenced the different 16S rRNA-ITS fragments, we compared the sequences to those deposited in nucleotide databases (NCBI) and we undertook a phylogenetic analysis for these distinct OTU's.

Materials and methods

*Collection of cyanobacterial mat samples***.** Material was collected from 2 distinct thermomineral drillings, Marghita and Roşiori (Bihor county, Romania). These drillings, because of the continuous water flow and natural light, allow mat formation as a blue-green layer attached to surface.

Genomic DNA extraction and purification. Genomic DNA was obtained using both the protocol detailed by Wu *et al.* (2000) and the "Wizard® Genomic DNA Purification Kit" (Promega). Humic acids (PCR inhibitors) were removed with aluminum sulfate (Dong *et al.*, 2006).

Amplification of 16S rRNA gene and ITS. The fragment 16S rRNA-ITS was amplified using specific cyanobacterial primers (Fig 1, Table 1). The PCR mix contained 10ul of 5x Green GoTaq Flexi Buffer, 3 ul of 25 mM $MgCl_2$, 1 ul of 10mM dNTP, 1 ul of 20 pmol 27F primer, 1 ul of 20 pmol ITER primer, 0,5 units of GoTaq Pol and water to a total volume of 50 ul. An initial denaturation at 94^0C for 5 min was followed by 30 cycles of denaturation (94^0C for 1 min), primer annealing (53^0C for 30 sec) and elongation (72^0C 1 min). The reaction was completed with a final step at 72^0C for 5 min.

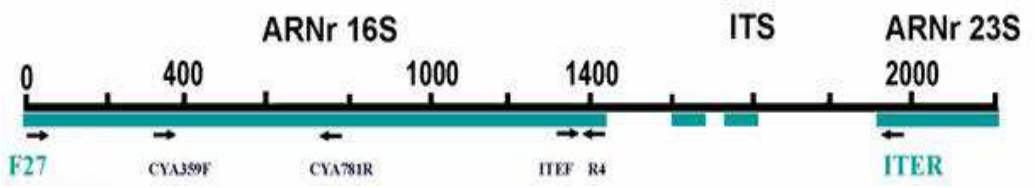


Fig. 1. A fragment of the cyanobacterial *rrn* operon and the annealing sites for the primers used in PCR reaction.

Table 1.

Primary structure of the primers used in this study

Primer	Sequence
16S 27F	5'-AGA GTT TGA TCC TGG CTC AG-3'
23S ITER	5'-CTC TGT GTG CCT AGG TAT CC-3'
16S CYA 359F	5'-GGG GAA TYT TCC GCA ATG GG-3'
16S CYA 781R	5'-GAC TAC TGG GGT ATC TAA TCC CWT T-3'
16S ITEF	5'-TGT ACA CAC CGC CCG TC-3'
16S R4	5'-TAC GGC TAC CTT GTT ACG AC-3'

Construction of cyanobacterial 16S rDNA-ITS clone library. The amplified 16S rDNA-ITS products were purified using "Wizard® SV gel and PCR Clean-Up System" (Promega) and ligated into pTZ57R/T plasmid (Fermentas). The resulted ligation products were used to transform *E. coli* J109 cells by electroporation. Clones were screened for α-complementation using X-Gal-IPTG on agar plates supplemented with ampicillin (100 mg/ml).

16S rDNA-ITS restriction map analysis. Plasmid DNA was extracted using **"**GeneJet™ Plasmid Miniprep kit" (Fermentas). Plasmids were used as template for the PCR reaction to reamplify 16S rDNA-ITS. Amplicons were digested using "FastDigest™ *Taq*I" (Fermentas) and the digestion products were migrated on a 1% agarose gel stained with ethidium bromide 0,5 µg/ml.

Based on electrophoretic profiles, we determined the distinct rDNA-ITS fragments. Cluster analysis based on estimated sizes of rDNA-ITS fragments which resulted after digestion was performed using StatGraphics.

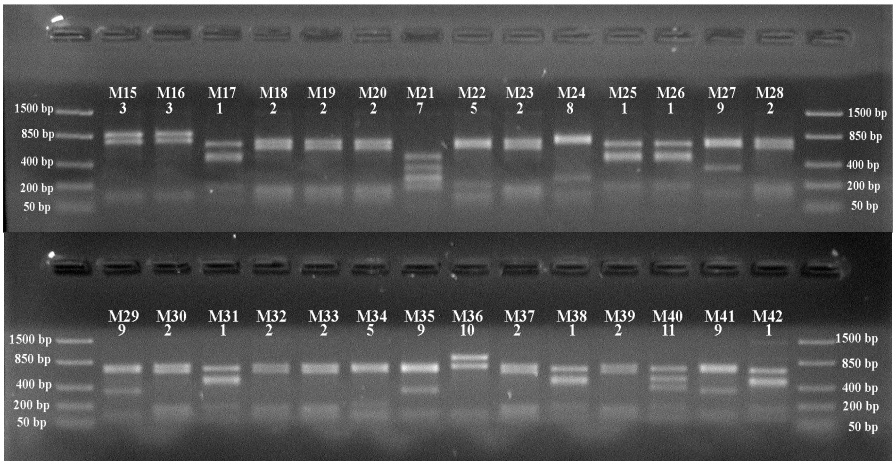
Sequence analysis. The different 16S rDNA-ITS fragments were sequenced on ABI Prism 310 Genetic Analyzer using 6 internal primers (Fig. 1, Table 1). The sequences obtained: a) were compared to the ones existing in the nucleotide databases using *blastn* algorithm (NCBI) for approximate determination of species from the analyzed mats; b) were used to perform a phylogenetic analysis in Phylip (Maximum Likelihood algorithm).

Results and discussion

After blue-white screening, a number of 46 colonies for Marghita and 40 colonies for Roşiori were selected. The PCR reaction with specific cyanobacterial primers (Fig 1, Table 1) using plasmids extracted from the selected colonies proved the existence of the target fragment in the plasmids.

Based on the electrophoretic profiles of the fragments resulted after digestion with *Taq*I (Fig. 2, Fig. 3), we observed the existence of 13 different rDNA-ITS fragments (13 distinct OTU's) in Marghita mat and only 2 rDNA-ITS fragments (2 distinct OTU's) in Roşiori mat (2 distinct OTU's).

Using the estimated size of restriction fragments we performed a cluster. The estimated lengths of restriction fragments were used for a cluster analysis for both Marghita and Roşiori mats (Fig. 4, Fig. 5). These clusters indicate the number of distinct OTU's in the two communities (13 and 2 respectively).Based on restriction maps we selected for sequencing the clones with different 16S rDNA-ITS profiles. We obtained partial 16S rDNA sequences which were compared with the sequences stored in public databases (NCBI GenBank) using *blastn* algorithm (NCBI) (Table 2).



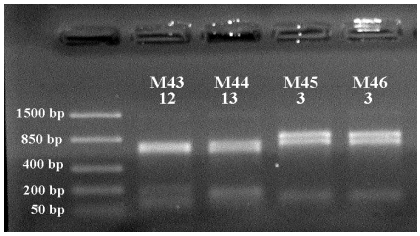


Fig. 2. The electrophoretic profile of the rDNA-ITS fragments from Marghita mat resulted after digestion with *Taq*I restriction endonuclease. The 13 different patterns are marked with numbers (1-13) written under the number of investigated clones (M1-M46).

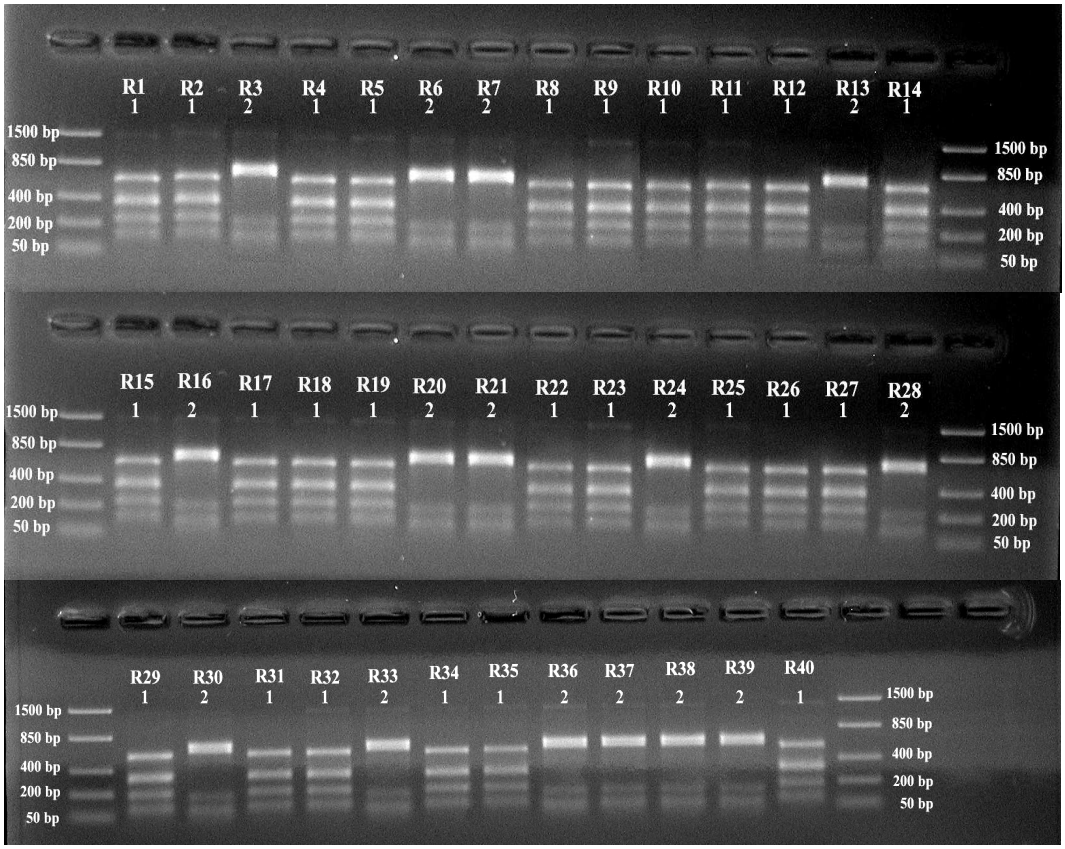


Fig. 3. The electrophoretic profile of the rDNA-ITS fragments from Roşiori mat resulted after digestion with *Taq*I restriction endonuclease. The 2 different patterns are marked with numbers (1, 2) written under the number of investigated clones (R1-R40).

BLAST interrogation revealed the fact that the primers supposed to be cyanobacterial specific can recognize other species, different from cyanobacteria (*Gemmatimonas*). Because of this, a further analysis (including phylogeny with cyanobacteria) on *Gemmatimonas* species (recently proposed as a distinct genus among bacteria) should be conducted to see the degree of relatedness between them and cyanobacteria species.

Table 2

Identity of 16S rDNA sequences obtained from community DNA of Marghita and Roşiori cyanobacterial mats

OTU nr.	Closest GenBank match	Identity %
MARGHITA		
M-OTU1	*Uncultured Gemmatimonadetes*	90
M-OTU2	*Leptolybgbya antarctica*	96
M-OTU4	*Phormidium pseudopristelyi*	98
M-OTU5	*Phormidium sp.*	96
M-OTU6	*Oscillatoriales cyanobacterium*	96
M-OTU12	*Uncultured Gemmatimonadetes*	91
M-OTU21	*Phormidium sp.*	97
M-OTU24	*Uncultured Gemmatimonadetes*	94
M-OTU29	*Microcoleus steenstrupii*	96
M-OTU36	*Uncultered cyanobacterium*	98
M-OTU40	*Uncultured Gemmatimonadetes*	88
M-OTU43	*Phormidium murrayi*	88
M-OTU44	*Uncultured Gemmatimonadetes*	89
ROSIORI		
R-OTU7	*Oscillatoriales cyanobacterium*	94
R-OTU18	*Uncultered cyanobacterium*	86

The phylogenetic tree (Fig. 6) emphasizes two things: the need of very good annotated sequences and repeated research before subscribing a certain sequence and the fact that the results could be influenced by Horizontal Gene Transpher. In our data there are cases when very good BLAST scores between two sequences do not reflect the same evolution. This is the case of *M-OTU43*, which is 88% similar to *Phormidium murryai*, but is located in the same evolution line with bacteria. Also *M-OTU29* is 96% similar to *Microcoleus steenstrupii*, but is in the same evolution line with *Symploca sp.* This sustains the idea that, at least for now, a molecular approach in ecological studies can be used for specie identification, but phylogeny of these microorganism is somehow uncertain.

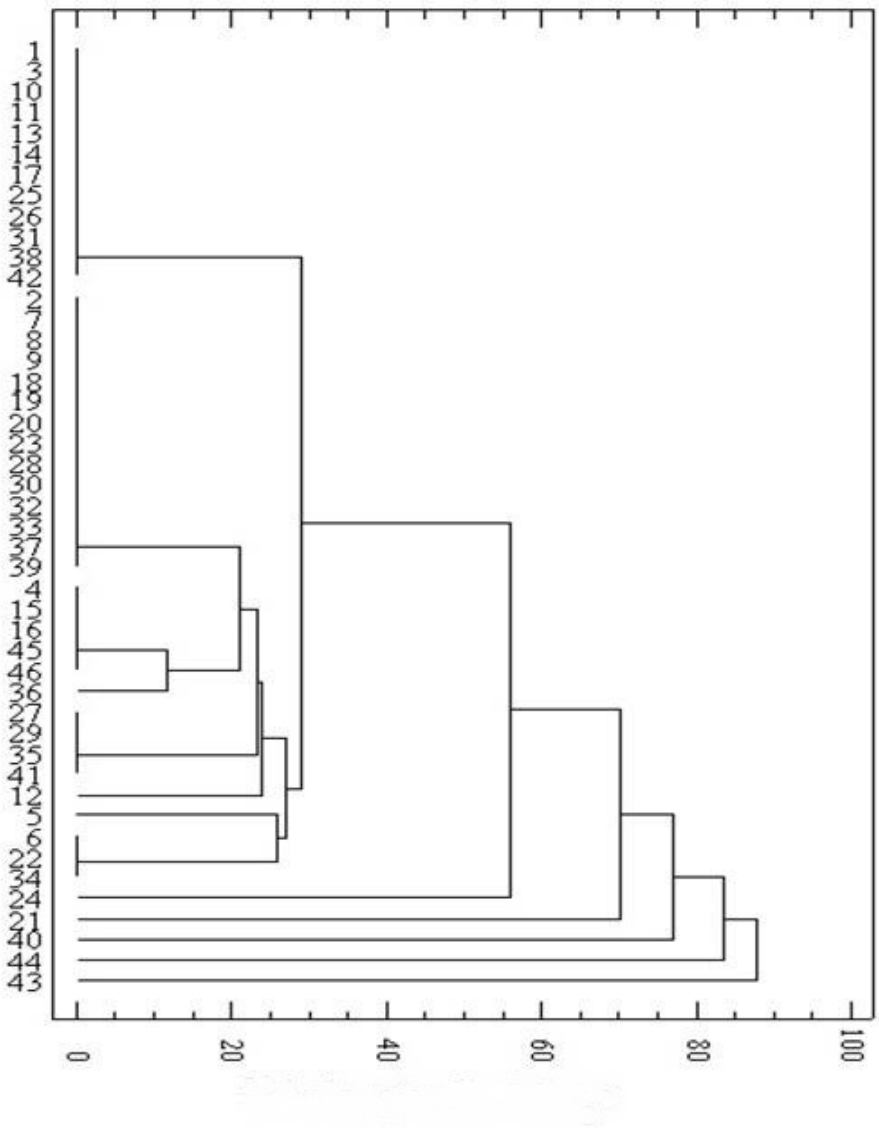


Fig. 4. Cluster analysis of the 46 clones from Marghita mat digested with *Taq*I endonuclease. The 46 clones form 13 distinct clusters, corresponding to 13 different OTU's. 0-100 scale represents the linkage distance.

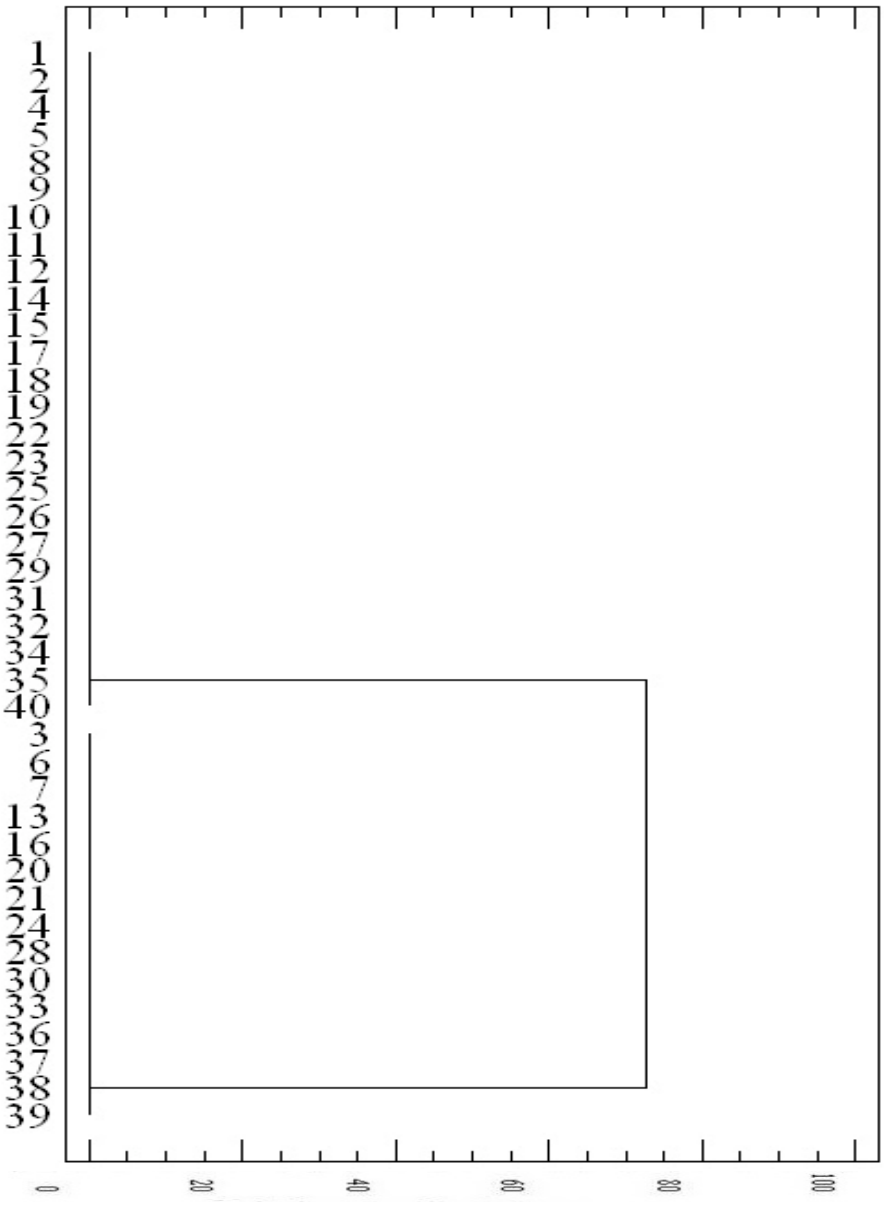


Fig. 5. Cluster analysis of the 40 clones from Roşiori mat digested with *Taq*I endonuclease. The 40 clones form 2 distinct clusters, corresponding to 2 different OTU's. 0-100 scale represents the linkage distance.

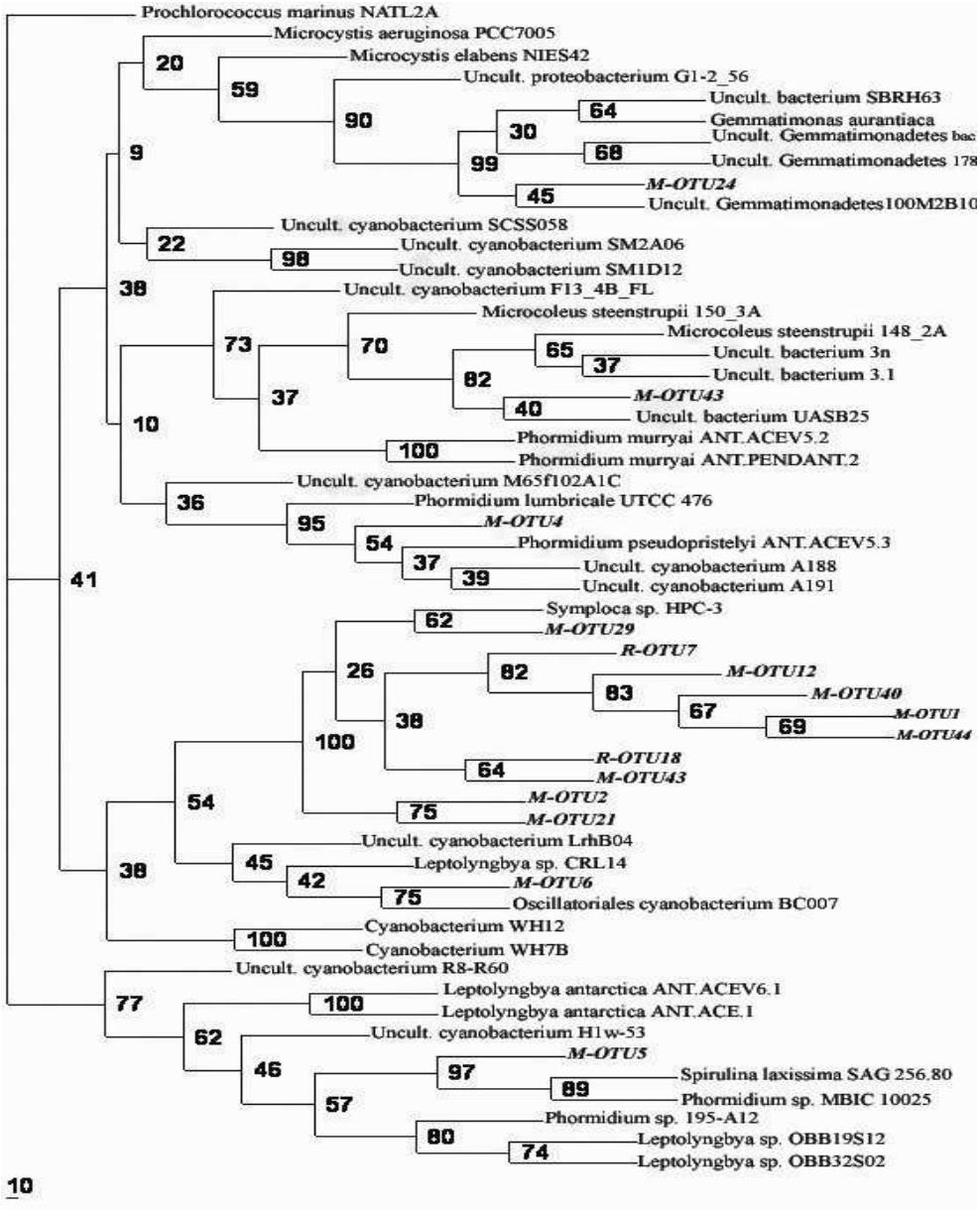


Fig. 6. Phylogenetic tree of Marghita and Roşiori OTU's constructed using Maximum Likelihood algorithm (PHYLIP- Felsenstein, 1989) on 16S rDNA sequences. Our own sequences are marked with bold italic. The numbers on the branches represent bootstrap values for 100 replicates.

Conclusions

In this study our objective was to investigate diversity and community structure of two cyanobacterial mats associated with thermomineral drillings: Marghita and Roşiori. We used for this purpose restriction map analysis, cluster analysis, sequencing and phylogeny reconstruction.

Restriction analysis using *Taq*I proved to be a good screening method. It can serve as a diversity estimation tool, because the number of distinct OTU's selected for sequencing using this method was not.

The identification of OTU's is highly dependent on the sequences existing in the nucleotide database. This is why repeated research and correct annotations is crucial for this type of study.

Our results brought valuable insights about the cyanobacterial diversity within the thermomineral mats from Margita and Roşiori. They represent also a solid proof of principle on how microbial diversity can be captured into an instant shoot at any point of community development. Our investigations will be refined in the future by using DGGE (Denaturing Gradient Gel Electrophoresis) of ITS fragments amplified from the entire community genomic DNA. This method, which combines the ability of PCR to amplify fragments from very small quantities of DNA with denaturing advantages, will provide a more accurate approach.

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EVALUATION OF THE FUNGICIDE EFFECT OF CERTAIN DISINFECTANTS USED ON DIFFERENT SURFACES IN FOOD INDUSTRY AND SANITARY INSTITUTES

ILDIKO-IREN RACKOVY[1], JÚLIA EDIT FEKETE[2], GYULA LÁSZLÓ FEKETE[3] and MIHAIL DRĂGAN-BULARDA[4]

SUMMARY. The efficiency of a disinfectant depends on its intrinsic biocide activity, the used concentration, time of contact, the nature of treated surfaces, hardness of water used to dilute the disinfectant, the organic substances present on the surfaces in question, the type and number of microorganisms present. Our goal was to study the fungicide effect of disinfectants in different concentrations, used for disinfection of surfaces in food industry and sanitary institutes, as well as to classify them according to their efficiency on fungi. To determine the fungicide effect of chemical disinfectants, we have used the method of surface test. We have also used a positive sample for comparison. We have determined the average survival, we computed the logarithmical reduction and the data have been processed with the Kruskal-Wallis statistic test. We have tested the effect of 8 disinfectants (P3-Topax 66, Omnicide, Terralin, Microzid AF, Javel Chloride, DJP-anios, DVA-anios, Suprasept) on the development of 5 types of fungi (*Candida albicans, Aspergillus niger, Penicillium sp., Mucor sp., Alternaria sp.*) on different surfaces (faience, glass, vinyl plastic, wood, stainless steel). We have found that their efficiency depends on the type of fungi and the tested surface. The efficiency of disinfection is decisively influenced by the chosen disinfectant and its concentration.

KEYWORDS: disinfectant, sanitation, fungi, surface test

Introduction

The effectiveness of a disinfectant depends on its intrinsic biocidal activity, the used concentration, the contact time, the nature of the surface disinfected, the hardness of water used to dilute the disinfectant, the organic substances present on the surfaces in question, the type and number of microorganisms present (Rackovy *et al.*, 2007.

Generally we do not know what kind of microorganisms are on the equipment and on the contaminated surfaces, therefore we have to use a suitable chemical disinfectant at a contact time and concentration which has a wide spectrum of activity in controlling the number and type of microorganisms.

[1] *Public Health Autority, Târgu-Mureş, Romania; E-mail: Rackovyildiko@freemail.hu*
[2] *Public Health Center Târgu Mureş, Romania; E-mail: feketeedit@yahoo.com*
[3] *University of Medicine and Pharmacy, Târgu Mureş. E-mail: dermafek@yahoo.com*
[4] *Babeş-Bolyai University, Department of Experimental Biology, Cluj-Napoca, Romania; E-mail: draganb@bioge.ubbcluj.ro*

Fungal cell wall appears to be a primary target site with postulated interaction with chitin. High concentration of disinfectant cause intracellular coagulation, degradation of proteins and nucleic acids. Microorganisms can adapt to disinfectant therefore the effectiveness of cleaning agents must be controlled periodically to minimize acquired resistance. Although the environment is a well-known source of human Aspergillus infection, Candida albicans survive well in the environment. Therefore, fighting against yeast reservoirs in the hospital environment would contribute to diminishing the risk of nosocomial infections (Wilson, 1986).

Our goal was to study the fungicide effect of disinfectants in different concentrations, used for disinfection of surfaces in food industry and sanitary institutes, as well as to classify them according to their efficiency on fungi.

Materials and methods

To determine the fungicide effect of chemical disinfectants, we have used the method of surface test, the simulated test of practical conditions. The test cultures used for investigations were chosen according to EN 14562: *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404 and funghy isolated from humans or the environment, strains whose survival in the hospital or on product contact surface is not admissible: *Penicillium sp., Mucor sp., Alternaria sp.*

A dilution of 10^3 organism per ml was made from each test culture. Phosphate buffer was used for dilution. 25cm^2 test areas were determined and designated for each disinfectant and for control in glass, stainless steel, tile, plastic, wood.

Sanitizers. Eight types of sanitizers were tested. They were diluted according to the manufacturer instructions. The active agent and recommended concentration of sanitizers are listed in Table 1. In the first step the maximum of recommended dilution of disinfectants (Terralin 0. 2-0. 5%, P3-Topax66 2-5%, Microzid AF 100 %, Omnicide 0. 2-0. 5 %, Quick javel 1%, Suprasept 0. 5%, Anios DVA, Anios special DJP)were used. Dilution was made with tap-water except Cidex and Microzid AF, which were used without dilution.

Sanitizer treatment. Volume of 0. 1 ml of test cultures were spot-inoculated on the determined and designated areas after cleaning with detergent, rinsing with tap-water, degreasing and disinfecting with 70 % ethanol. Each test-area was inoculated with approximately 10^3 test organisms/25 cm^2. The surface was air-dried at room temperature for 30 minutes. After drying 0. 2 ml of diluted disinfectants were inoculated in each test-area. Exposure time was 30 minute while disinfected surfaces were dried just like in the industrial and hospital practice. Control-areas were not treated with any disinfectant. Sampling was made with contact media (Tripticaza soia agar-TSA) by analogy with common hygienic control practice. Contact media contains a range of neutralizing agents to inactivate the disinfectants (lecithin and polysorbat 80).

After proper incubation (*Candida albicans:* 23±2 °C, 48-72 hours; *Aspergillus niger:* 23±2°C, and the other fungi for 5 days) the number of survived cells (CFU/25 cm^2) was compared to the control.

The efficiency of disinfectants was evaluated by the formula: LRV = log N_0/N where LRV is the logarithmic reduction value of CFU, N_0 is the initial and N is the final number of organisms (CFU/25 cm^2). The efficiency of disinfectants was accepted when: LRV ≥ 2. Percentable reduction value of CFU was used for statistical evaluation which was carried out with Kruskal Wallis software (ANOVA).

Table 1.

Disinfectants tested against test-organisms by surface-test

Disinfectant	Recommended concentration	Active agents
P3-Topax66	2-5 %	Potassium-hydroxide, Sodium-hypochlorite, Non-ionic surfactant
Terralin	0. 2-0. 5 %	Dimethylbenzil – alchil- amoniumchloride, fenoxipropanol, Non-ionic surfactant
Suprasept	0. 5 %	Glutaraldehyde
Microzid AF	Undiluted	Ethanol, Propanol
Omnicide	0. 2-0. 5 %	Glutaraldehyde, Dimethylbenzil-coco-amoniumchloride
Quick javel	1 %	Sodium- diclorizocianurat Sodium-carbonate, Adipic acid
Anios DVA	Undiluted	Didecil-dimetil-amoniu-chloride, phenol, glycine and alcohol
Anios special DJP	Undiluted	Aldehyde, Didecil-dimetil-amonium-chloride

Results and Discussion

We have taken 220 surfaces test. According to the result of the surfaces test the 0. 2% of Omnicide was effective on each studied surfaces against *Penicillium sp*., *Alternaria sp*, but *Candida albicans* and *Aspergillus niger* were present (4-5 UFC) on glass, wood and stainless still surfaces. Omnicide 0. 2% reduced fungal load by only 1 to 2 $\log_{10}$ yeast cell ml(LRV<2). Only 0. 5%Omnicide maintained its fungicidal activity, reduced fungal load on every surfaces by more than 4 $\log_{10}$ yeast/mould cells ml. Good fungicide properties of Omnicide are the result of strong antimicrobial action of aldehydes. They cause protein and enzyme denaturation and membrane damage of microbial cell (see Fig. 1).

Quaternar ammonium compounds are considered as bound biocides (Burge *et al*.,1989) They are attached chemically to the surface providing a permanent effective dose during sanitation (Kemper and White, 1991). In spite of this fact the

results of Terralin in 0. 2% was reduced fungal load by only 1 $\log_{10}$ yeast/mould cells ml. on every surfaces.

In the repeated test increased concentration (0. 5%) was used, which was effective, the number of test organisms was reduced by at least 2 log cycles on every surface. The investigations of Theraud et al. (2004) presented similarly low effectiveness of quaternar ammonium compounds against fungi.

According to the result of the surfaces test the 2% of P3- TOPAX 66 was not effective against *Penicillium sp*., (7-9 UFC / on every surface) nor *Candida albicans* on wood and stainless still surfaces. P3- TOPAX 66 was effective against all test organism in 5% solution.

Pasanen and co-workers (1997) reported that hypo-chlorite based products provided the best biocidal effect, preventing fungal growth on dusty sheet metal (Fig. 2).

Microzid AF has a lower biocidal effect on the viability of the test organisms, reduced fungal load by only 1 to 2 $\log_{10}$ yeast/ mould cell ml, on every surface survived 3-4-5 cells, *Candida albicans* on wood, plastic and stainless steel (LRV<2) surface, just like *Aspergillus niger, Penicillium sp., Mucor sp. and Alternaria.*

Quick Javel is a chlorine based disinfectant. It had little effect against *Aspergillus sp.* and *Penicillium sp.*, but *Candida albicans* strains showed no growth after 2 and 7 days of incubation. Fungi are less sensible then yeast cells to chlorine based disinfectant (Passanen et al., 1997).

According to the result of surface test, anios DJP, was ineffective against every test organism.

We have determined the survival average of test cultures in present of disinfectants on different surfaces which was 1. 23%.

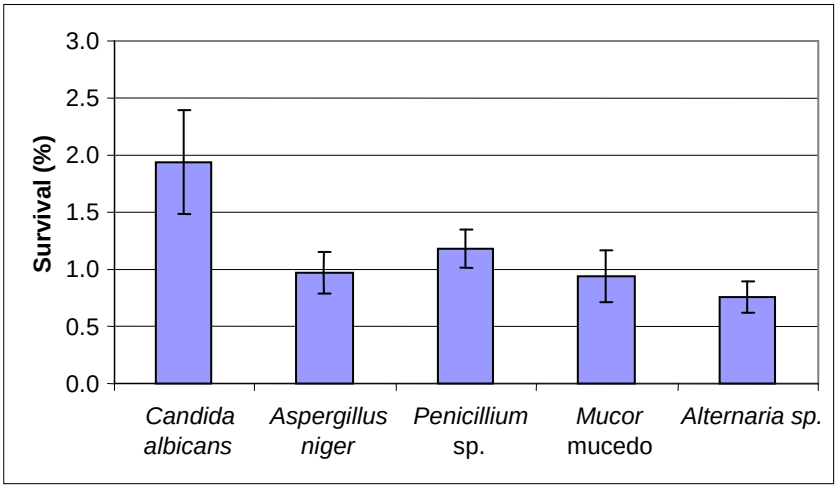

Fig. 1. The survival average of fungi in presence of disinfectants.

We have found out that *Candida albicans* has a survival rating much higher then the other 4 fungi cultures. On the other hand we could not demonstrate a significant difference between the 5 fungi survival rating with the Kruskal-Wallis test (p=0. 2).

The survival of fungi depending from the texture of the surface on which they are developing presented in the figure 2.

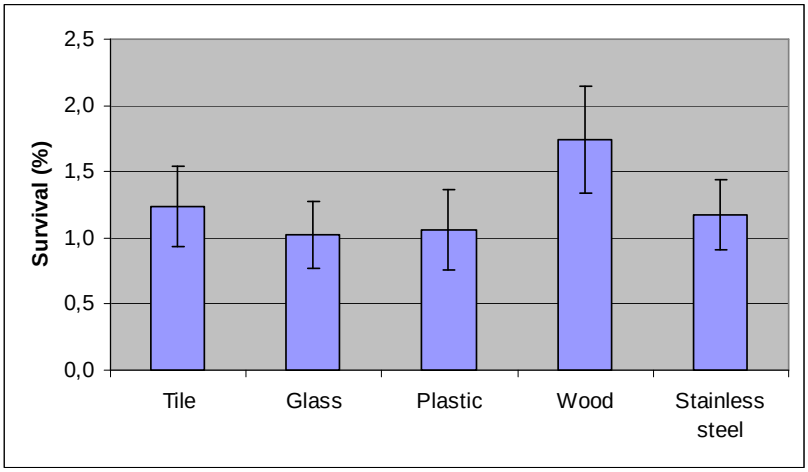

Fig. 2. *The survival average depending of the tested surface in the presence of studied disinfectants*

The Kruskal-Wallis test showed a significant statistic difference (p=0. 05): more fungi colonies survived on the wood surface after using the disinfectants then on other studied surfaces.

The rates of fungi survival were grouped according to the disinfectants studied.

Table 2.

Fungi survival depending on studied disinfectants

Desinfectant	Survival %	Standard deviation	Standard error
P3 Topax66 2%	1. 17%	0. 006991	0. 001563
P3 Topax66 5%	0. 31%	0. 005448	0. 001218
Omnicid 0. 2%	1. 12%	0. 008425	0. 001884
Omnicid 0. 5%	0. 18%	0. 002604	0. 000582
Terralin 0. 2%	1. 44%	0. 003946	0. 000882
Terralin 0. 5%	0. 45%	0. 001596	0. 000357
Microzid AF cc.	1. 33%	0. 005492	0. 001228
Javel Chlor	0. 44%	0. 004566	0. 001021
DJP anios	**6. 05%**	0. 038790	0. 008674
DVA anios	0. 60%	0. 004662	0. 001042
Suprasept 0. 5%	0. 44%	0. 005572	0. 001246

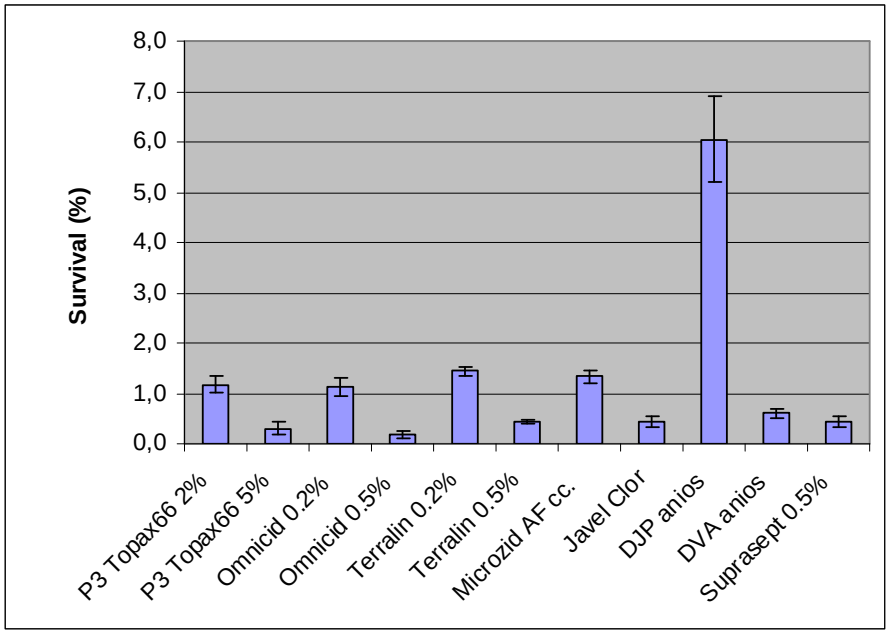

Fig. 3. *Survival average depending of disinfectant used*

DJP-anios and Microzid AFcc. were least effective then every other studied($p<0.001$).

In average, the DJP disinfectant could only destroy 6% of fungi. It was observed that after a while of depositing, in the liquid there were appearing filiform precipitates, and the fact that can be explained by the low efficiency of this product.

Products P3 Topax 66, Omnicide and Terralin in concentration of 5% respectively 0. 5% were 1-5 times more effective then the 2% respectively the 0. 2% concentration.

Satisfactory results gave Javel chlor, DVA-anios and Suprasept 0. 5%, too. The best result was delivered by Omnicid 0. 5%, here the survival average was only 0. 18% reported to the control test.

The efficiency of disinfectant on different surfaces is also shown in Fig. 5.

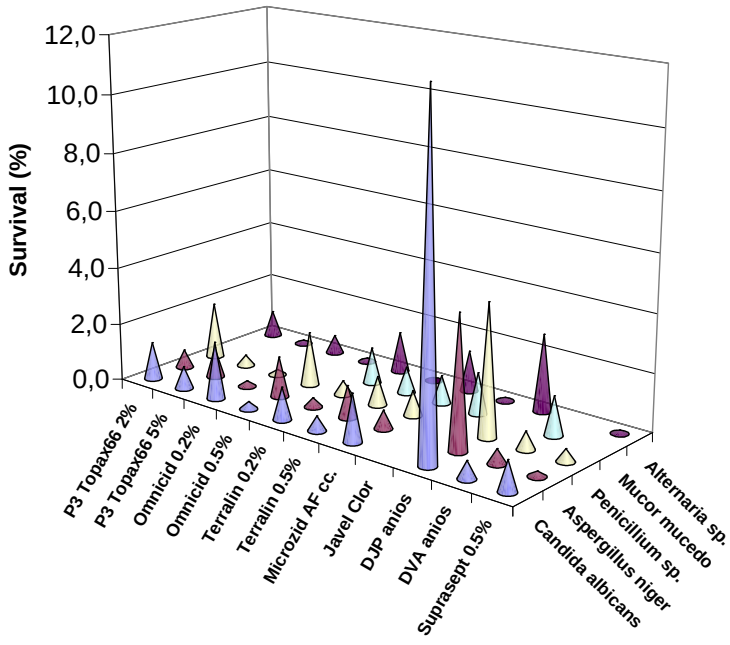

Fig. 4. *The efficiency of disinfectants on fungi groups- totalized dates*

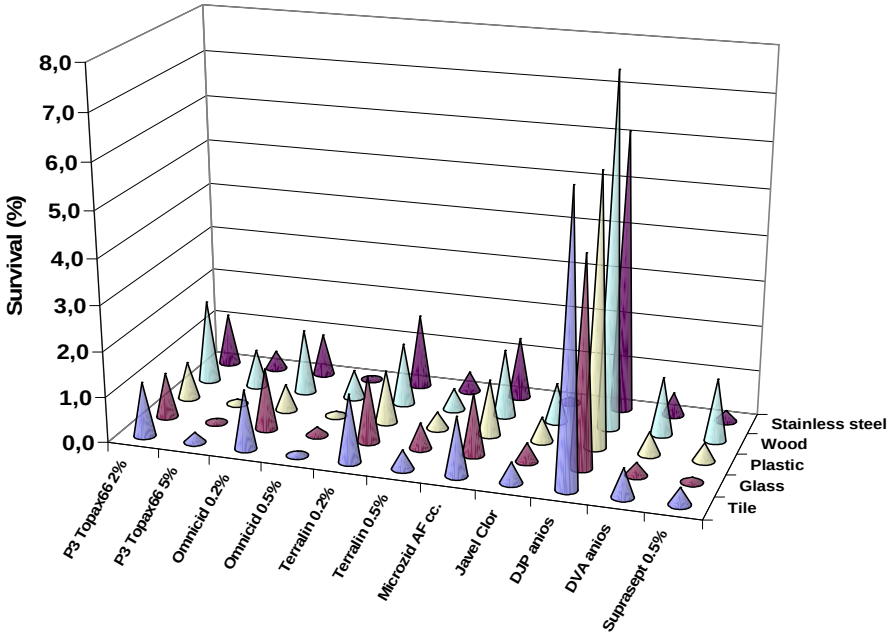

Fig. 5. *The efficiency of disinfectants depending from type of surface*

Conclusions

1. There were tested 8 disinfectants (Terralin 0. 2-0. 5%, P3-Topax66 2-5%, Microzid AF 100 %, Omnicide 0. 2-0. 5 %, Quick javel 1%, Suprasept 0. 5%, Anios DVA, Anios special DJP undiluted) on 5 kinds of fungi growth (*Candida albicans*, *Aspergillus niger*, *Penicillium sp.*, *Mucor sp.*, *Alternaria sp.*) on different surfaces (tile, glass, PVC, wood, stainless steel), and found out that their effective depends of the type of fungi and the tested surface.

2. The pathogen yeast - *Candida albicans* - seems to be the most resistant microorganism against the most of disinfectants comparing to the other kinds of moulds tested.

3. The P3 Topax 66, Omnicide and terralin disinfectants manifested the most important biocide activity, and DJP-anios proved to be the least efficient.

4. 5% concentration P3 Topax 66 used only in the milk processing factory proved that it destroys most of fungi on stainless steel and tile surfaces comparing to wood surface.

5. The 2% concentration Omnicide was ineffective, only high concentration of this product had fungicidal activity. The low concentration is not accepted for use in the health care units.

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OLIGASE AND POLYASE ACTIVITIES IN VARIOUS TYPES OF SOILS FROM PARÂNG MOUNTAINS

RAHELA CARPA[1], MIHAIL DRĂGAN-BULARDA[1]

SUMMARY. Five soil samples from the Parâng Mountains in the South-Eastern part of the Hunedoara county, from five altitudinal vegetation zones (the alpine zone, the subalpine zone, the conifers zone, beech zone and the Maleia flood plain) were collected and these samples have been analyzed qualitatively enzymologically.
In the soil samples, the following enzymatic activities have been qualitatively determined: four oligase activities: maltase, saccharase, lactase and cellobiase and three polyase activities: amylase, dextranase and inulinase. The studied activities were detected in all the 5 altitudinal vegetation zones, with differences noticed only in the intensity of the processes. Generally, the highest intensity of qualitative enzymatic activities were registered in soil samples from the conifers vegetation zone.

KEYWORDS: soil samples, oligase activities, polyase activities

Introduction

Studies on enzyme activities in soil are important as they indicate the potential of the soil to support biochemical processes which are essential for the maintenance of soil fertility (Brzezinska *et al*., 1998).

The enzymatic activity of soil is the result of the activity of accumulated enzymes and of the enzymes of the proliferating microorganisms. The accumulated enzymes are considered to be enzymes present and active in a soil where the proliferation of microorganisms does not occur. The sources of the accumulated enzymes are the microorganisms cells, but they can also come from the vegetal and animal organic residues. The enzymes are accumulating in the soil as free enzymes (freed from the live cells and from the disintegrated cells) and also as enzymes bound to the cellular constituents (enzymes present in the disintegrated cells), in the live but not proliferating cells. The enzymes of the proliferating microorganisms are enzymes freed from the live cells under multiplication and enzymes which are found within the cells undergoing the multiplication process (Drăgan-Bularda and Paşcu, 1997).

There is a series of factors affecting the enzymatic activities in soil. The ecological parameters such as seasonal changes, geographic location or water regime may affect the level of the enzymatic activity by influencing both the yielding of enzymes by plants and microorganisms in the soil and their persistence under natural

[1] *Faculty of Biology and Geology, Babeş-Bolyai University,1 Kogălniceanu Str, 400084/Cluj-Napoca, Romania, E-mail: k_hella@yahoo.com; E-mail: draganb@bioge.ubbcluj.ro*

conditions. The high content of mineral and humic colloides is often associated with the stable but less active enzymes. That is why the factors influencing or changing the physical and chemical properties of the soil may probably affect the imobilization, stability and catalytic activity of the soil enzymes (Gianfreda and Bollag, 1996).

Thus, the enzymatic activity analysis offers, in a shorter time than microbiological analysis, the suggestive data regarding the processes that are taking place in soils or in other natural habitats. The analysis of the enzymatic activity in soils of the mountainous ecosystems is a research method in evaluation the functional diversity of the microbiota involved in the biogeochemical cycles (Schloter *et al*. 2003).

Studies regarding the assessment of the mountainous soil were previously carried out in the Vladeasa Mountain (The Apuseni Mountains) (Nemeş *et al.,* 1977). Complex studies (ecological, eco-pedological, enzymological) were also carried out on soils from National Retezat Park and they were completed by a synthetical approach (Paşca *et al.,* 1993).

For this study the soil samples were collected from 5 altitudinal vegetation zones in the Parâng Mountain (the alpine zone, the subalpine zone, the conifers zone, beech zone and the Maleia flood plain). Located South-East of the Petroşani town, the Parâng Mountains form the eastern barrier of the Jiu Valley, with a large surface of 1100 km^2. These are the tallest mountains in the area, and the second tallest mountains in Romania after the Făgăraş Mountains in the East and the fifth highest peak (The Parângul Mare Peak) after the Peaks Moldoveanu, Negoiu, Viştea Mare, Călţun (Popescu, 1986).

In order to complete the ecology studies existing in the specialty literature (Doniţă *et al*., 2005) regarding the vegetation zones in the Parâng Mountain and to understand the operation and composition of the nitrogen-fixing microbiota existing within these types of soil it is necessary to perform the microbiological and enzymological analysis of the soil specific to the each mountain zones. We specify that microbiological and enzymological information related to the soils in the Parâng Mountain are not mentioned in the specialty literature. The present paper analyze for the first time the evolution of the qualitative enzymatic activities from the mountainous soils in Parâng, which is necessary because the enzymological analyses pursue the knowledge of the percentage of some enzymes involved in the biogeochemical cycles of the elements and the achievement of an overall image of the biological activity in the soil ecosystems.

Material and Methods

Five types of soil from different vegetation zones in the Parâng Massive were studied in the autumn of 2007. In table 1 there are presented the analyzed soils and some of their physical-chemical characteristics.

Table 1.

Soils in the altitudinal vegetation zones from Parâng Mountains

No.	Sampling zone	Soil type	pH	Eh (oxido-reducing potential) (mV)	Humus (%)	Total N values
1	Alpine	Humosiosol (humic-silicate)	4.40	146	32.96	1.058
2	Subalpine	Cryptopodzolic	4.13	160	24.36	0.529
3	Conifers	Podzolic	3.73	181	28.50	1.020
4	Beech	Eutric cambosol	5.31	110	4.93	0.270
5	Flood plain	Pelic fluvisol	6.20	75	4.18	0.133

The soil samples were taken from 15-20 cm depth and the sampling sites of the soil were: the alpine zone (2216 m), the subalpine zone (1871 m), the conifers zone (1646 m), beech zone (1286 m), and the Maleia flood plain (805 m).

The following enzymatic activities were qualitatively estimated in the soil samples: four oligase activities – maltase (α-glucosidase) (MA), saccharase (invertase) (SA), lactase (β-galactosidase (LA) and cellobiase (β-glucosidase) (CeloA) and three polyase activities: - amylase (AA), dextranase (DA) and inulinase (inulase) (IA). The above-mentionated enzymatic activities were chosen because their determination in the soil samples constitutes a research tool to evaluate the functional diversity of the microbiota involved in the biogeochemical cycles of the elements.

The technique used to establish these enzymatic activities was paper circular chromatography. The reaction mixture consisted of 3 g soil + 2 ml toluene (for preventing the proliferation of microorganisms) + 5 ml 2% enzymatic substrate (maltose, saccharose, lactose, cellobiose, starch, dextrane and inulin); incubation: 7-14 days at 37°C. After developing the chromatographic paper, the reductive hydrolytic products were emphasized. The larger spots for the hydrolytic products show the higher activities of the oligase and polyase (Drăgan-Bularda, 2000).

Results and Discussion

The oligases (SA, MA, LA and CeloA) activities are well represented qualitatively in all the five soil samples.

The presence of the saccharase (SA) in soil could be correlated with the microbial activity and could be used as a “fertility index” (Gianfreda and Bollag, 1996). As one may notice in the chromatogram in fig. 1, the saccharase activity is very intense in all five soil samples collected from the five vegetation zones as compared to the control sample, which was proved by the occurrence of an intense glucose spot. This indicates that the vegetal residues incorporated in the soil and transformed by the microorganisms have led to the synthesis of the saccharase under the action of the specific substrate.

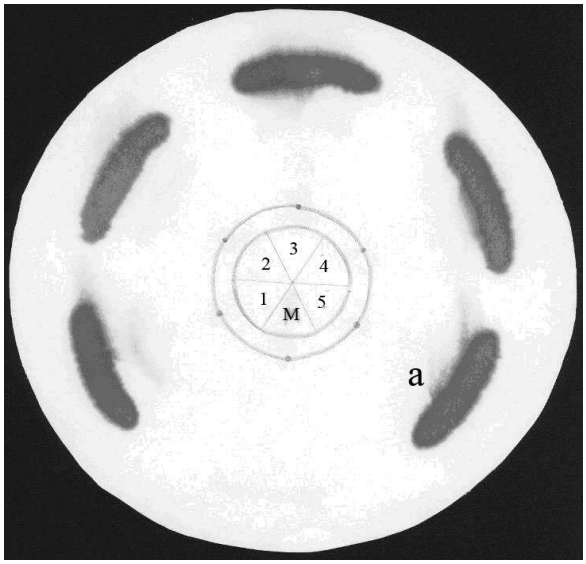

Fig. 1. Saccharase activity (SA).
1-5: soil samples (see Table. 1) + enzymatic substrate (saccharose 2%).
M = control - saccharose solution 2%.
a = glucose spot.

One can notice that the level of the saccharase activity in all the five soil samples is similar, indicating that the accumulated saccharase constitutes one of the key enzymes in the carbon cycle. Our analyses show once more that the saccharase accumulated in the mountain soils has a high level, just like the fertile arable soils (Nemeş *et al*., 1997).

The cellobiase activity is illustrated in fig. 2. One can notice that this is relatively significant in all the soil samples analyzed comparatively with the control-sample. The cellobiose is a disaccharide resulting from the degradation of the cellulose, representing a sign of its transformation in the mountain soils. The cellobiose proved to has of the maximum intensity in sample 3 (the coniferous zone), as the entire added substrate was hydrolyzed in glucose (the cellobiose spot has disappeared from point *a*) as compared to the control-sample. In all the soil samples analyzed the enzymatic substrate was not entirely hydrolyzed. Of the 4 samples where hydrolyze was partial, the weakest intensity was noticed in sample 5 (soil in the Maleia flood plain).

In addition, the cellobiase activity (β-glucosidase) (CeloA) is stimulated by different ions. The hydrolysis speed became higher through addition by different cations like nitrates. The heavy metals (Ag, Cu and Hg) acting like reversible inhibitors (Vasilescu, 1961).

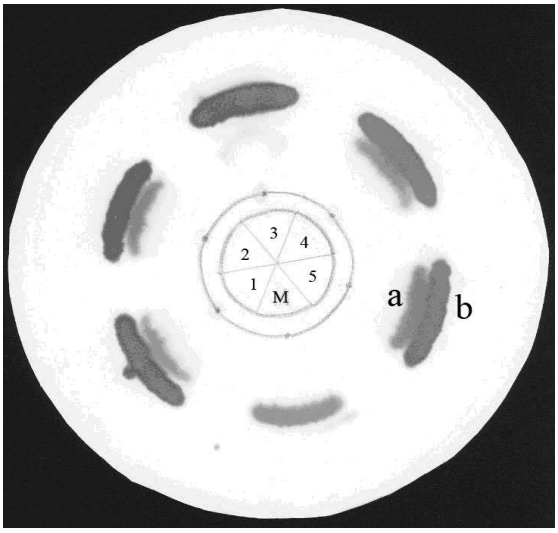

Fig. 2. Cellobiase activity (CeloA).
1-5: soil samples (see Table 1) + enzymatic substrate (cellobiose 2%).
M = control - cellobiose solution 2%.
a = cellobiose spot.
b = glucose spot.

The cellobiase is another oligase involved in the carbon circuit in nature and represents the sign of enzymatic transformation of the cellulose under the action of the accumulated cellulose. The presence of the cellobiase as an accumulated enzyme represents a significant proof regarding its accumulation in soil as a result of the cellulose residues degradation; the cellulose is the most important vegetal polysaccharide.

The chromatogram in fig. 3 represents spots of the maltase activity. One can observe that the activity was maximum in 4 soil samples (1, 2, 3, 4), in the soil samples from the Maleia flood plain (5) the maltose was not entirely hydrolyzed (a). The low maltase activity existing in sample 5 is probably related to the nature of the existing vegetation in that area where prevail the species of alder tree, patience, nettles and other weeds specific to flood plains in the mountain area which influence probably the enzyme activity. The maltase activity of the soil reflects the transformation of the starch in the vegetal residues by the intermediary maltose-glucose stage.

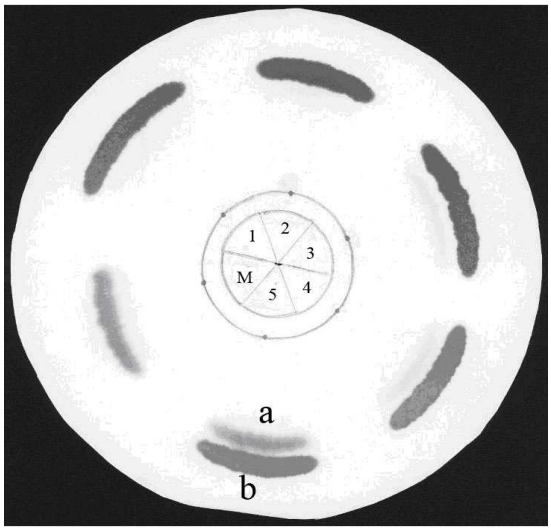

Fig. 3. Maltase activity (MA).
1-5: soil samples (see Table 1) + enzymatic substrate (maltose 2%).
M = control - maltose solution 2%.
a = maltose spot.
b = glucose spot.

The lactase activity was also present in the analyzed samples (fig. 4). The sample 3 (the coniferous zone) presented the most intense lactase activity, which was proved by the disappearance of the lactose spot (total hydrolyze) from the point *a*. The other samples have a significant lactase activity but without recording the total hydrolysis of the lactose spot.

The presence of the lactase in the mountain soil samples leads to the conclusion that these do not have a high enzymatic level as also in the arable soils the lactase activity is less intense than the saccharase and maltase ones.

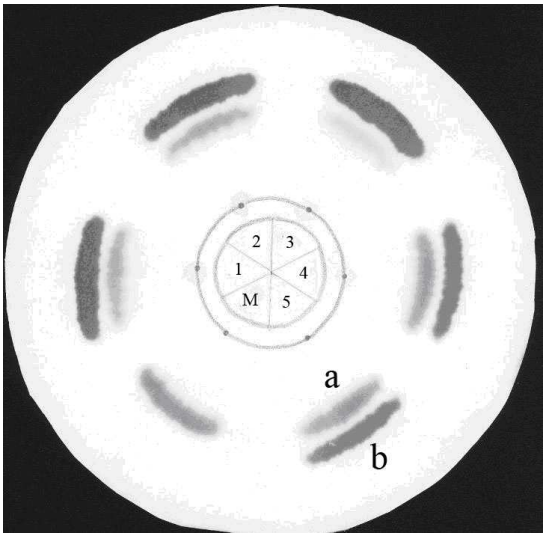

Fig. 4. Lactase activity (LA).
1-5: soil samples (see Table 1) + enzymatic substrate (lactose 2%).
M = control - lactose solution 2%.
a = lactose spot.
b = glucose spot.

The polyase (polysaccharidase) activity of soils represents the main component of the enzymatic potential of a soil, as the polysaccharides represent the most important substances in the vegetal residues reaching into the soil. Of course, for the synthesis of the soil enzymes there also contribute the saccharides reached in the soil as a result of the roots secretion (Kiss *et al.*, 1975). The polyase (AA, DA and IA) activities were also well represented qualitatively in all soil samples.

Among the analyzed polysaccharides activities the most important proved to be the amylase activity (fig. 5). The amylase activity (AA) increases proportionally with humus content and with capacity of cationic change (Eliade *et al.*, 1975). It is interesting that from the analysis of the chromatogram in fig. 5 one can observe that the amylase activity was very intense in all the soil samples without significant differences according to the humus quantity which ranged very much depending on the vegetation zones.

Thus, the sample in the alpine zone (1) indicated a high percentage of humus 32.96 of the moder type of alpine meadow and in the samples from the coniferous zone (3) the humus percentage is high (28.5). Here we meet a raw type of humus (of the mor type); then sample 2 is next (from the sub-alpine) where the humus content (moder of alpine meadow) is high (24.36). In sample 4 (the beech zone) and in the sample 5 (of meadow) the forest mull type humus, with a weakly to moderately acid reaction, it reaches a low percentage of 4.93, respectively 4.18.

It is known that the microorganisms are the ones which degrade the vegetal residues up to simple compounds and also, starting from the simple compounds, they synthesize the organic substances of the soil (the humus). A lower amylase activity cannot always be correlated with a lower humus content.

One can notice that as a result of the starch hydrolysis, glucose is produced as a final product of reaction without any other intermediary compounds (oligosaccharides or maltase).

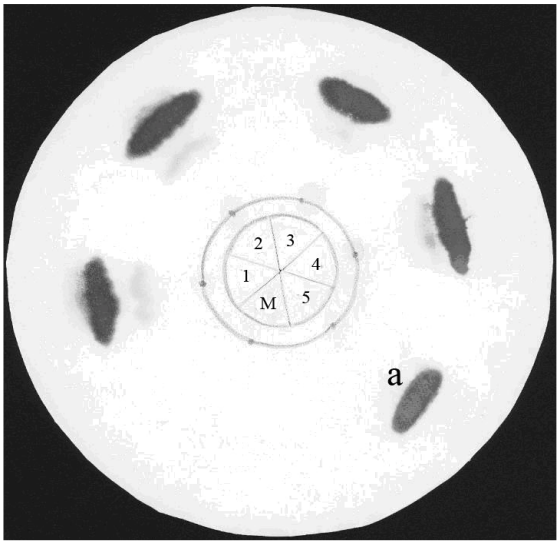

Fig. 5. Amylase activity (AA).
1-5: soil samples (see Table 1) + enzymatic substrate (starch 2%).
M = control - starch solution 2%.
a = glucose spot.

Another polyase (polysaccharidase) highlighted in the soil samples was the dextranase which hydrolyzes the dextran (fig. 6). The dextran is a polyglucoside which is not strictly related to the vegetal residues. The synthesis of the dextran into the soil is achieved by microorganisms starting from the saccharose under the action of the dextran-sucrase (Drăgan-Bularda and Kiss, 1972 (a). The dextran plays an essential role in the aggregation of the soil particles, its synthesis reflecting the complexity of the soil microbiota. The hydrolysis of this polyglucoside under the action of the dextranase was proved by studies performed on brown forest soil and on chernozem (Drăgan-Bularda and Kiss, 1972(b).

The dextranase activity was presented in all the soil samples studied. Differences in its intensity were noticed. The most intense activity was recorded in the samples 3 (the coniferous zone), 1 (the alpine zone) and 4 (the beech zone) followed by the samples 5 (the meadow) and 2 (the sub-alpine zone) as compared to the control-sample.

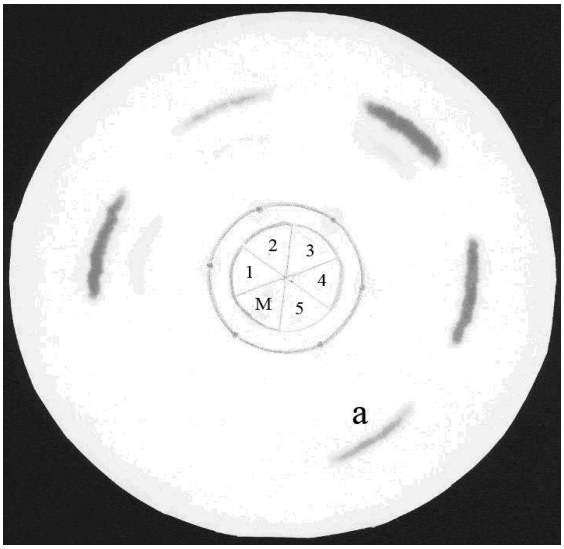

Fig. 6. Dextranase activity (DA).
1-5: soil samples (see Table 1) + enzymatic substrate (dextran 2%).
M = control - dextran solution 2%.
a = glucose spot.

The oscillations regarding the intensity of the dextranase activity cannot be justified by the vegetal residues reached into the soil, as this depends more on the presence and the activity of the dextranolytic microorganisms in the mountain soil samples.

The polysaccharidase activity highlighted was the inulinases activity (fig. 7). The inulinase (IA) is a polysaccharidase which specifically hydrolyses the inulin (polyfructoside). This activity is closely connected to the presence of the vegetal residues which reach into the soils rich in this polyfructoside (Drăgan-Bularda and Paşcu, 1997). From the analysis of the chromatogram in fig. 7 one can notice that only in two soils (samples 2 and 3) the hydrolysis of the inulin was total (the inulin spot disappeared from the start point *a*) as compared to the control sample where, at the start point only the inulin spot appears, without any other impurifying substance. In the other soil samples (1, 4 and 5) the inulin was not totally hydrolyzed, which was proved by the occurrence of the inulin spot at the start point, more or less intense.

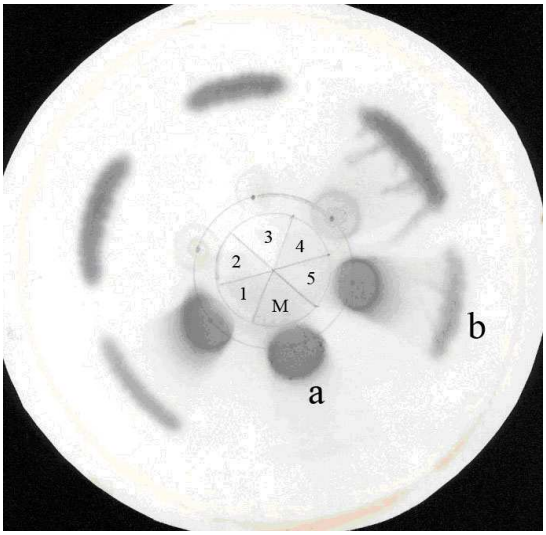

Fig. 7. Inulinase activity (IA).
1-5: soil samples (see Table 1) + enzymatic substrate (inulin 2%).
M = control - inulin solution 2%.
a = inulin spot.
b = glucose spot.

Conclusions

All the seven enzymes (four oligases and three polyases) studied qualitatively have shown variations of their intensities, depending on the sampling place and on the necessary substrate for the enzymes synthesis by the microorganisms.

The oligase activities (maltase, saccharase, lactase and cellobiase) were significant in all the analyzed 5 soil samples. The maximum intensity was registered in the sample 3 from the coniferous zone. A weak intensity was presented by the Maleia flood plain sample (5) where, probably, the vegetation type existing here has influenced the studied oligases activities.

The polyase activities (amylase, dextranase and inulinase) are well represented in all the soil samples.

The amylase activity was very intense in all the soil samples, without significant differences according to the humus quantity which varied much depending on the vegetation zones.

The dextranase activity was present in all the analyzed soil samples.

The differences between the samples, as regard the intensity of the enzyme activity was justified by the presence and activity of the dextranolitic microorganisms activity in the mountain soil samples.

The inulinase activity presented a high level in all the soil samples. The most intense activity was registered in samples 2 (the sub-alpine zone) and 3 (the coniferous zone) where the hydrolysis of the inulinase was total.

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