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Aquatic fungi growing on substrates containing chitin[1]

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The chitinophilic fungi in various types of water bodies (slough, ponds, lakes and rivers) was studied with reference to the chemical environment. The wings of flies, dragonfly and crayfish carapace were used as bait. Fifty-six species of chitinophilic fungi were found in various types of water bodies.

INTRODUCTION

One of the substrates in water which is attractive to some aquatic fungus species is chitin. It is present in the higher fungi and above all in such arthropods as crustaceans and insects. The carapace of crustaceans and the cuticle of insects consist mainly of chitin and other important component proteins (K a r l s o n, 1980). In respect of crustaceans, in each body of water smaller (plankton) or larger crustaceans are present which on dying provide a substrate for chitinophilic fungus species. Their exuviae of insects, found in quite large numbers in the littoral zone of waters and mature insects which happen to be in the water by accident provide a substrate for these fungi.

While conducting our studies on aquatic fungi belonging to various physiological groups, we decided to investigate the group of aquatic chitinophils with reference to the chemistry of water. It is known that the presence of substrate is not sufficient for the development of some fungi species. However, the physical and chemical properties of the substrate play a important role in the growth of fungi (R e i s e r t, F u l l e r, 1962; U n e s t a m, 1966; S p a r r o w, 1968).

MATERIALS AND METHODS

The studies carried out in the north-eastern part of Poland in the years 1990--1992 included a bog, 2 ponds, 11 rivers of various sizes, 4 small forest lakes

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("suchary") and 34 lakes of different limnological types (C z e c z u g a, 1993). The water was collected in 5-litre Ruttner bucket from the depth at which the bucket was immersed. In the water, the temperature was measured and the following determinations were made: the pH, CO_2, oxygen dissolved, the oxidability of the water and its alkalinity, the hardness of the water calculated in Ca and Mg, the concentration of ammonium, organic nitrogen, nitrate, phosphates, chlorides, iron, sulphates, dry residue and substances dissolved in water and the suspension in water. For the determination of the different chemical elements in the water the methods recommended by Standard Methods (G o l t e r m a n, C l y m o, 1969) were employed; the details of these methods were described in a previous paper (C z e c z u g a, P r ó b a, 1980).

The water samples for the mycological studies from each of the sites were transported in sterile glass containers of 1.5 litres capacity. In the mycological laboratory, they were placed in sterilized beakers, (capacity of 0.6 l), to which the appropriate baits were added in accordance with the general principles of culture (F u l l e r, J a w o r s k i, 1986).

The wings of flies (*Sarcophaga carnaria*), dragonfly wings (*Aeschna juncea*) and a crayfish carapace (*Orconectes limosus*) were used as bait. The insect exuviae collected in spring from the surface of the water during the period of flights of masses of ephemerids, chironomids and mosquitoes were examined. The wings and the carapace were boiled several times with the water being changed each time. They were then cut into small pieces and placed as bait in the containers with water from the various water bodies studied. The samples thus prepared from each site were kept in the laboratory for 3-6 months and precautions were taken to ensure that the thermolighting conditions were as close as possible to those prevalent outside the laboratory. The fungi found were determined by their morphological features, measurement being made the shreds, oogonia, and oospores by means of an ocular microscope. Species of the chitinophilic fungi were determined from the mycological keys of S k i r g i e ł ł o (1954), S p a r r o w (1960) and B a t k o (1975).

RESULTS

The results of chemical studies of water are presented in Table 1 and 2 (only data for the water reservoirs not yet published are included in the Table 1). The data reveal wide range of trophicity in the water reservoirs studied, definied by the content of phosphorus and various forms of nitrogen. The values of this and other parameters were within the range of variability of waters of dystrophic lakes (suchary--small forest lakes), typical eutrophic (Lake Nieciecz) and typical oligotrophic lake (Lake Hańcza).

In the water bodies investigated the presence of 56 aquatic fungus species growing on substrates containing chitin was determined (Table 3). Some of the species developed on all the baits whereas others grew only on some of them.

Table 1

Chemical composition of the water in particular water bodies ($mg\ l^{-1}$)*

Specification	Lake			Suchar		
	Szelment	Klusy	Rajgrodzkie	II	III	IV
Temperature °C	13.4	14.6	11.9	15.4	15.5	15.2
pH	8.25	8.54	7.58	6.14	5.82	5.34
Oxidability	10.5	14.63	7.4	13.8	10.2	19.6
CO_2	15.4	19.8	24.2	13.2	14.3	22.0
Alkalinity in $CaCO_3$ (in mval l^{-1})	4.0	2.7	3.4	0.4	0.2	0.2
$N(NH_3)$	0.02	1.020	0.315	0.75	0.46	1.09
$N(NO_2)$	0.013	0.003	0.006	0.004	0.003	0.003
$N(NO_3)$	0.040	0.065	0.080	0.080	0.0	0.010
PO_4	2.960	0.550	1.335	0.010	0.0	0.0
Cl	36.0	49.0	43.0	34.5	35.0	39.0
Total hardness in Ca	72.0	48.24	29.52	2.88	2.50	3.65
Total hardness in Mg	24.96	17.20	35.26	2.15	0.0	0.0
SO_4	32.09	27.97	32.91	9.87	4.94	9.05
Fe	0.30	0.10	1.08	0.0	0.0	0.0
Mn	0.10		0.08			
Dry residue	371.0	334.0	362.0	31.0	14.0	46.0
Dissolved solids	349.0	317.0	341.0	16.0	12.0	35.0
Suspended solids	22.0	17.0	21.0	15.0	2.0	11.0

*The chemical analysis of the water from: Lakes Bezymienne, Guber, Ławki, Pogubie Wielkie, Ryńskie, Śniardwy, Tałtowisko and Tałty see C z e c z u g a (1991 c, 1993); River Biała, Lake Necko and pond Branicki Palace see C z e c z u g a and M u s z y ń s k a (1994); River Biebrza see C z e c z u g a et al. (1990); Lake Hańcza and River Czarna Hańcza see C z e c z u g a et al. (1990); River Horodnianka and River Narew see C z e c z u g a and P r ó b a (1987); pond Grażyna see C z e c z u g a et al. (1988); River Pisa and River Skroda see C z e c z u g a (1991 d); River Rudawka see C z e c z u g a and M u s z y ń s k a (1993); River Węgorapa see C z e c z u g a (1991 b); Lake Wigry see C z e c z u g a (1991 a).

Table 2

Chemical composition of the water in Lake Elk and River Płoska in some month (mg l^{-1}) (1990)

Specification	Lake Elk				River Płoska			
	February	May	August		February	May	August	October
Temperature °C	0.5	14.8	18.6	5.4	0.2	12.4	17.2	6.0
pH	7.6	8.2	7.7	8.1	7.6	7.9	8.0	7.8
Oxidability	8.4	13.4	10.8	7.1	7.4	5.6	5.6	7.4
CO_2	12.1	0.5	0.0	6.6	12.6	8.8	13.2	8.8
Alkalinity in $CaCO_3$ (in mval l^{-1})	3.6	3.4	2.8	3.7	4.2	3.1	2.6	3.6
$N(NH_3)$	0.0	0.07	0.30	0.24	0.32	0.28	0.04	0.22
$N(NO_2)$	0.0	0.008	0.0	0.002	0.010	0.015	0.003	0.008
$N(NO_3)$	0.0	0.01	0.0	0.0	0.0	0.04	0.01	0.03
PO_4	0.31	0.05	0.01	0.80	0.96	0.54	1.30	1.29
Cl	36.0	35.0	64.0	25.0	17.0	16.0	19.0	21.0
Total hardness in Ca	58.32	56.16	43.92	41.04	59.76	58.32	57.60	61.20
Total hardness in Mg	17.20	22.36	18.49	20.64	13.62	8.60	13.33	12.90
SO_4	25.92	23.84	26.33	31.68	40.76	36.61	24.27	55.95
Fe	0.20	0.0	0.0	0.0	0.30	0.45	0.35	0.25
Mn	0.10	0.02	0.0	0.10	0.05	0.15	0.08	0.10
Dry residue	276.0	333.0	305.0	232.0	334.0	280.0	258.0	227.0
Dissolved solids	271.0	282.0	241.0	228.0	314.0	251.0	253.0	209.0
Suspended solids	5.0	51.0	64.0	4.0	20.0	29.0	5.0	18.0

Table 3

Aquatic fungi growing on different substrates containing chitin in various water bodies

Species	Flies wings	Dragonfly wings	Insect exuviae	Crayfish carapace	Bodies of water	
					Lakes	Rivers
Chytridiomycetes						
Allomyces arbuscula Butler	x	x	x	.	x	.
Asterophlyctis irregular Karl.	x	.	.	.	x	x
A. sarcoptoides Petersen	.	.	x	.	x	.
Catenaria anguillulae Sorokin	x	.	.	.	x	x
C. verrucosa Karling	x	x	.	.	x	.
Catenophlyctis variabilis (Karl.) Karl.	x	.	.	.	x	.
Blastocladiella britannica Horest. Cant.	x	x	.	.	x	x
Blastocladiopsis parva Whiffen	x	x	.	x	x	.
Chytriomyces aureus Karl.	.	.	x	.	x	.
Ch. hyalinus Karl.	x	x	.	.	x	.
Ch. poculatus Willoughby et Townley	x	x	.	.	x	.
Diplophlyctis complicata (Willogh.) Batko	.	x	.	.	x	.
Rhopalophlyctis sarcoptoides Karl.	.	.	x	.	x	.
Karlingia chitinophila Karling	x	x	.	.	x	.
Karlingiomyces asterocystis (Karl.) Sparrow	.	.	.	x	x	.
K. dubius (Karl.) Sparrow	.	.	.	x	x	.
Obelidium mucronatum Nowak.	x	x	.	x	x	x
Phlyctorhiza endogena Hanson	.	x	x	.	x	x
Phlyctochytrium aureliae Ajello	x	x	.	x	x	.
Polychytrium aggregatum Ajello	x	x	.	.	x	.
Rhizoclosmatium auranticum Sparrow	.	.	x	.	x	.
R. globosum Petersen	.	.	x	.	x	.
R. hyalinum Karl.	.	.	x	.	x	.
Rhizidium chitinophilum Sparrow	.	x	.	x	x	.
R. nowakowskii Karl.	.	.	.	x	x	.
R. ramosum Sparrow	.	.	x	.	x	.
R. verrucosum Karl.	.	x	.	.	x	x
Rhizophlyctis petersenii Sparrow	.	x	.	x	.	x
Rhizophydium amoebae Karl.	.	x	.	.	x	.

Cont. of Table 3

Species	Flies wings	Dragonfly wings	Insect exuviae	Crayfish carapace	Bodies of water	
					Lakes	Rivers
Siphonaria petersenii Karl.	.	.	x		x	.
S. variabilis Peters.	x	x	.		x	x
Zygochytrium auranticum Sorokin	.	.	x		x	.
Hyphochytriomyces bivellatus Nabel	x	.	.		x	x
R. hansonii Karl.	.	.	x		x	.
Oomycetes						
Achlya dubia Coker	.	x	.		x	x
A. klebsiana Pieters	x	.	x		x	x
A. oligacantha de Bary	x	.	x	x	x	.
A. prolifera Nees	.	.	.	x	x	x
Aphanomyces amphigynus Cutter	x	.	x	.	x	.
A. astaci Schikora	x	x		x	x	x
A. bosminae Scott	.	.	.	x	x	.
A. irregularis Scott	x	x	x	x	x	x
A. laevis de Bary	.	.	.	x	x	.
A. stellatus de Bary	.	x	x	.	x	.
Apodachlya brachynema (Hildebr.) Pringsh.	x	x	x	.	x	x
Apodachlyella completa (Humphrey) Indoh	x	.	.	.	x	.
Cladolegnia unispora (Coker et Couch) Johan.	x	.	.	.	x	.
Isoachlya torulosa (de Bary) Cejp	x	x	x	.	x	x
Saprolegnia ferax (Gruith) Thurnet	x	x	.	.	x	.
Leptolegnia caudata de Bary	x	x	.	x	x	x
Leptomitus lacteus (Roth) Agardh	x	.	.	x	x	x
Pythium jirovecii Cejp	.	.	.	x	x	.
Zygomycetes						
Zoophthora conica (Nowak.) Batko	x	.	x	.	x	.
Z. curvispora (Nowak.) Batko	.	.	x	.	x	.
Z. rhizospora (Thaxter) Batko	.	.	x	.	x	.
Deuteromycetes						
Aspergillus candidus Link	x	x	.	x	x	.
Number of species	29	26	22	18	55	18

Such species of aquatic fungi as *Asterophlyctis irregularis, Rhizidiomyces bivellatus, Apodachlyella completa* and *Cladolegnia unispora* grew only on the fly wings whereas *Diplophlyctis complicata, Rhizidium verrucosum* and *Achlya dubia* developed only on dragonfly wings. The remaining species developed only on insect exuviae collected from the area i.e.: *Asterophlyctis sarcoptoides, Chytriomyces aureus, Rhizoclosmatium aurantiacum, R. globosum, Rhizoclosmatium hyalinum, Rhizidiomyces hansonii, Rhizidium ramosum, Siphonaria petersenii, Zygochytrium aurantiacum* and two species of the genus *Zoophthora: Z. curvispora* and *Z. rhizospora*. The crayfish carapace proved to be the attractive substrate only for *Karlingiomyces asterocystis, K. dubius, Rhizidium nowakowskii, Achlya prolifera, Aphanomyces laevis and Phytium jivorecii*. On the other hand, on all four types of substrates containing chitin only *Aphanomyces irregularis*, one of the commonest chitinophilic fungi in this area, developed. In general, most fungi grew on the wings of flies (29) and dragonfly (26), fewer on insect exuviae (22). The lowest number of fungi was found on crayfish carapace (18 species).

There were differences in the occurrence of chitinophilic fungi in water (rivers) and stagnant (lakes) waters (Table 3). In addition, there were seasonal changes in the number of various species of fungi (Table 4) were noted.

Table 4

Chitinophilic fungi in lake Ełk and river Płoska in particular months

Species	Lake Ełk	River Płoska
Chytridiomycetes		
Asterophlyctis irregularis Karling		X
Phlyctochytrium aurelaie Ajello	III, X	
Siphonaria variabilis Peter.	V	
Oomycetes		
Achlya klebsiana Pieters		II, XII
A. oligacantha de Bary	III, IV	
A. prolifera Nees		I
Aphanomyces astaci Schikora	X	
A. irregularis Scott	I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII	I, II, III, IV, V, VI, VII, IX, XI, XII
A. brachynema (Hild.) Pringsh.	X	X
Cladolegnia unispora (Coker et Couch) Johannes	II	
Leptomitus lacteus (Roth) Agardh		I, II, III
Zygomycetes		
Zoophthora conica (Nowak.) Batko	VI	

DISCUSSION

The present investigations showed that among the species of aquatic fungi growing on substrates containing chitin (wings, aquatic insect exuvia and the crayfish carapace) there were some species which had been found to be chitinophils. However, in our investigations we observed the growth of fungi, which to date had not been reported to be chitinophilous, on a substrate containing chitin. These are *Saprolegnia ferax, Leptomitus lacteus, Blastocladiopsis parva, Aspergillus candidus, Catenaria anguillulae, Catenaria verrucosa, Catenophlyctis variabilis, Chytriomyces poculatus* (Fig. 1-3). The *Saprolegnia ferax*, is aquatic fungus in the waters of north--eastern Poland (C z e c z u g a, 1994). In our case it developed on fly and dragonfly wings in the water collected from a small forest lake IV. As is known, within this species several varieties are distinguished (C e j p, 1959). *Leptomitus lacteus* is a nitrophilic fungus which occurs in water containing municipal wastes and is also found on the spawn of some species of fish (C z e c z u g a, W o r o n o w i c z, 1993). *Blastocladiopsis parva*, on the other hand, has been found to date in different types of waters ranging from springs and rivers to various kinds of lakes (C z e c z u g a, 1991a-c; C z e c z u g a et al., 1989; C z e c z u g a, W o r o n o w i c z, B r z o z o w s k a, 1990). It has also been noted on substrates containing keratin (C z e c z u g a, M u s z y ń s k a, 1994). *Aspergillus candidus*, is (U h l m a n n, 1975) a fungus which infects humanus. Within this species a number of subspecies and varieties have been distinguished. *Aspergillus niger* is known to grows on substrates containing chitin (O t a k a r a, 1964). *Catenaria anguillulae, C.verrucosa, Catenophlyctis variabilis* and *Chytriomyces poculatus*, on the other hand, were also noted on substrates containing keratin (C z e c z u g a, M u s z y ń s k a, 1994). *Catenaria anguillulae* has so far been found on "insects" and eggs of *Arachnida* (S p a r r o w, 1960). It is possible that the growth of these four species of fungi on substrates containing chitin other fungi known to be chitinophilic "sevre" a specific phase of succesion of reduces on a given substrate. In addition to chitin, protein also plays an important role in the composition of the intergument of arthropods, part (K a r l s o n, 1980). *Aphanomyces astaci* grew on the wings of flies and dragonfly in the waters of Lake Ełk, River Biebrza and small forest lake II. This species is known to be the first and foremost parasite of crayfish of the genus *Astacus* (S c h i k o r a, 1903; R e n n e r f e l t, 1936; S c o t t, 1961; U n e s t a m, 1965, 1968; T r a c y, V a l l e n t y n e, 1969) and frequently causes the complete destruction of population of noble crayfish in a various water bodies (R a h e, S o y l u, 1989). *Aphanomyces bosminae* Scott. also grow on the carapace of *Bosmina coregoni* Baird in the water of Lake Ełk.

Some of the chitinophilic fungus species identyfied only sporadically occurred during the two years' study. These were *Allomyces arbuscula, Chytriomyces hyalinus, Karlingia chitinophila, Polychytrium aggregatum, Rhizidiomyces bivelatus, Rhizidium chitinophilum* and *Cladolegnia unispora*. *Allomyces arbuscula* occurred only in small forest lake IV in Suwałki Province in autumn. These are small shallow lakes in forests with acid water and low mineral salt content. In small forest lake III *Chytriomyces hyalinus* also occurred in autumn. It is also known to be a chitinophilic fungus (M o o r e, M i l l e r, 1973) and in our study it grew only on the fly and dragonfly wings.

Similarly, *Karlignia chitinophila* grows on substrates containing chitin (B a t k o, 1975). In the present studies it occurred only in the water of Lake Rajgród in autumn where it was also found on the wings of flies and dragonflies.

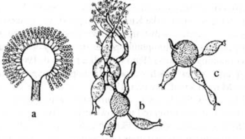

Fig. 1. *Aspergillus candidus* (a – head of the conidiosphores with chains of spores); *Catenaria anguillulae* (b – policentric thallus, c – sporangium from intersporangium bridges 7-29 x 9-63 μm)

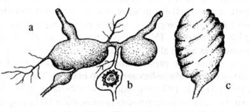

Fig. 2. *Catenaria verrucosa* (a – thallus, b – spore 14-22 μm); *Chytriomyces poculatus* (c – empty sporangium 10-35 x 7-32 μm)

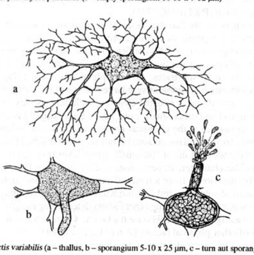

Fig. 3. *Catenophlyctis variabilis* (a – thallus, b – sporangium 5-10 x 25 μm, c – turn aut sporangium)

The species *Polychytrium aggregatum* (A j e l l o, 1948), not only grow on chitin but also on substrates containing cellulose. In our studies it grew on the wings used as bait in the water of Lake Klusy and Rajgród. We observed the development of this fungus on cellulose substrates in Lake Wigry and some of the nearby lakes (C z e c z u g a, 1991a). Chitinophilic species frequently develop at the same time on substrates containing cellulose (F u l l e r, B a r s h a d, 1960; U n e s t a m, 1966) though there are species which are only chitinophilic, e.g. such as *Karlingiomyces asterocystis* (M u r n a y, L o v e t t, 1966).

Rhizidiomyces bivellatus is a saprophyte, which grows on insect exuviae (N a b e l, 1939). It was observed to grow only on fly wings in the waters of Lake Rajgród. *Rhizidium chitinophilum*, like *Rhizidiomyces bivellatus*, occurred in Lake Rajgród only in autumn where it grew on dragonfly wings and the crayfish carapace. Worthy of note is the presence of *Aphanomyces laevis* in the waters of Lake Pierty in Suwałki Province. It developed on crayfish carapace. It is known (S k i r g i e ł ł o, 1954; B a t k o, 1975) to be not only an aquatic but also a soil saprophyte as well as a facultative parasite of crayfish and algae. It also often grows on dead insects and fish. On the other hand, *Cladolegnia unispora* was isolated from the fly wings in the water of Lake Ełk in February. This fungus is not only capable of growing on dead insects but also on twigs and is also found in loam and at the bottom of drying water bodies usually in spring. The present studies showed that several fungus species grow on exuviae of insects whose larvae develop in water. These fungi contribute to (S p a r r o w, 1937; D i c k, 1970) the redution of substrates containing chitin. Such substrates as exuviae occur in large amounts in some water bodies especially in spring. The substrates containing chitin, which fall to the bed, particularly plankton crustacean carpaces, can also be materialized by chitinolytic bacteria such as *Pseudomonas chitinovorum* (P a l u c h, 1973).

There is no doubt that the presence of substrate containing chitin is not sufficient for the development of various chitinophilic fungi since a environmental factors also play an important role. This confirmed by the fact that a given fungus frequently grows in one body of water but does not grow in a nearby reservoir even though the latter contains chitin substrates. An example of this is Lake Rajgród and the small forest lakes in which a comparatively large number of chitinophilic species occurred in comparis on with lakes situated close by, including species which occurred only in Lake Rajgród and other small forest lakes. Lake Rajgród is of mesotrophic type and the data regarding the chemical analysis of the water did not appear to differ significantly from the data obtained from the other lakes. However the chemical analysis of the water of the of "the small" forest lakes revealed that all the "Suchary" were of acidic character. In particular, small forest lake IV (pH 5.34) had a high oxygen consumption, a large amount of carbon dioxide, and ammonia nitrogen and a low content of sulphates and calcium while there was absolutely no magnesium or iron in the water. Data regarding other factors determining the growth of chitinophilic fungi, such as light or shade (H o r e n s t e i n, C a n t i n o, 1961, 1962) as well as a number of other physical factors (A n t i k a i j a n, 1949; W i l l o u g h b y, 1959; K a r l i n g, 1967) were definied. It is possible that they determine the development of these fungi in Lake Rajgród. It is known that some species are to be found in both an aquatic and a land habitats. In the present investigations such species of chitinophilic fungus were among others, *Karlingiomyces dubius* (Willoughby, 1957) isolated from in crayfish carapace the water of Lake Szelment Wielki.

The investigations of seasonal changes in the number of chitinophilic fungi occurred in summer. This would be in agreement with our earlier observations concerning other physiological groups of zoosporus fungi (Czeczuga, 1991 a) – even the representatives of *Hyphomycetes* (Czeczuga, Orłowska, 1993).

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