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Species diversity of keratinophilic fungi in various soil types

Research Article

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Abstract: Keratinophilic fungi are a highly specialized, keratin-degrading ecological group. They live in natural environments, mostly in the keratin-rich remains of dead animal in the soil. We investigated species diversity in four types of soils with different physico-chemical properties. The strain material was identified based on morphological characters. Different representatives of *Chrysosporium* and geophilic dermatophytes dominated depending on soil pH. Geophilic dermatophytes were represented by one species, *Trichophyton ajelloi*, and the *Chrysosporium* group was represented by *Chrysosporium keratinophilum*. The frequency of *Trichophyton ajelloi* increased with an increase in pH, and it reached the maximum in strongly acidic soil (podzol), unlike the *Chrysosporium* group. The frequency of *Chrysosporium keratinophilum* was positively correlated with the content of humus, nitrogen, $CaCO_3$ and phosphorus in the soils.

Keywords: *Soils • Geophilic dermatophytes • Chrysosporium • Trichophyton ajelloi*

1. Introduction

Keratinophilic fungi are a highly specialized group of fungi that have evolved an ability to degrade native keratin. Some species of keratinophilic fungi cause dermatophytoses and are parasites of keratinized tissues in humans and animals. Epidemiological chains of such fungi comprise humans, animals and the environment. Anthropophilic dermatophytes, such as *Trichophyton rubrum*, spread from person to person. Others, such as *Trichophyton verrucosum*, are zoophilic dermatophytes that cause infections in animals and can be transmitted to humans. Representatives of both groups can survive exosomatically, e.g. in the soil, on "pieces" of the host's keratin structures such as hair, or epithelia, without losing infection abilities. Other keratinophilic fungi are saprotrophs living in the keratin-rich remains of dead animals deposited in the natural environment. They comprise fungi known as geophilic dermatophytes and the species of the genus *Chrysosporium* related to them. Otčenašek and Dvořak [1] distinguish three groups of species within geophilic dermatophytes: naturally non-pathogenic fungi represented mostly by *Trichophyton terrestre* Durie *et.* Fray and *T. georgiae* Varsavsky *et.* Ajello, often pathogenic fungi, *i.e. Microsporum gypseum* (Bodin) Guiard *et.* Grigoriakis and *M. fulvum* Uriburu, and accidentally pathogenic fungi, including *Microsporum cookei* Ajello and *Trichophyton ajelloi* (Vanbreuseghem) Ajello.

Geophilic dermatophytes and keratinolytic *Chrysosporium* species participate in the recycling of carbon, nitrogen and sulphur of native keratin in the soil and other keratin-containing environments. Representatives of both groups use keratin as the only source of C, N, S and energy, releasing large amounts of $N\text{-}NH_4$ and $S\text{-}SO_4$ as end products [2]. These fungi therefore constitute an important group of destruents of the organic substance in the soil. Keratin remains (feathers, hair, *etc.*) found in the soil or on its surface are not only a valuable substrate for keratinophilic soil fungi but also a specific environment that allows them to survive and protect themselves against other competitive saprotrophic microorganisms [3].

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Keratinophilic fungi have weak saprotrophic competition abilities. Their frequency in the soil is relatively low and they are sporadically isolated from the soil when the dilution plate method is used. They can be effectively isolated using hairbaiting. The method, also known as the ToKaVa method, was described independently by three authors: Thoma, Karling, Vanbreuseghem, and is known under their joint names [4].

Trichophyton ajelloi (previously *Keratinomyces ajelloi*) was the first geophilic dermatophyte isolated from the soil with method by Vanbreuseghem [5]. *T. ajelloi* and *M. gypseum* are two most widespread geophilic dermatophytes in the soil worldwide [6]. The geographic distribution of the two species differs, which is consistent with the types of biotopes colonized by them. The thermotolerant *M. gypseum*, which reaches the growth maximum at 38°C [7], more often colonizes soils of subtropical and tropical areas, especially on both American continents [6]. The typically mesophilic *T. ajelloi*, which does not grow at 37°C, dominates in Europe [6].

Monitoring studies of these species in European soils (Germany, Poland) conducted in the 1960s showed that the frequency of *T. ajelloi* did not fall below 60% and that of *M. gypseum* did not exceed 10% of the total frequency of geophilic dermatophytes [6]. Relatively recent studies Kornikowicz-Kowalska and Bohacz [8] on the occurrence and the distribution of geophilic dermatophytes in the soils of central and eastern Poland showed similar quantitative relationships between these groups of keratinomycetes. Of a total of 1319 strains of geophilic dermatophytes, 1022 represented *T. ajelloi* and 132 represented *M. gypseum*, which corresponded to 77.5% and 10% of the frequency of the species. *M. gypseum* mostly colonizes soils with near-neutral pH, *T. ajelloi* mostly occupies acidic soils [7,9]. The frequency of *T. ajelloi* in the soil increases as pH decreases and becomes a monocultre in strongly acidic soils, and decreases when soil pH increases [8].

Geophilic dermatophytes and *Chrysosporium* species related to them exhibit a high variability of physiological properties associated with keratinolytic abilities. In Kornikowicz-Kowalska [2,10] studies on the keratinolytic activity of 34 strains of these fungi showed that the parameters of native keratin degradation (substrate mass loss, release of peptide substances and ammonia, sulphate production and pH changes – alkalinization) were characterized by a considerable total variation (Cv). With the exception of pH (Cv 4-7%), total variation values were high and often reached 50% [10]. They were caused by the strain diversity in 95-98%.

The diversity of the keratinolytic activity showed affinity with the origin of a strain. Higher activity was observed in strains isolated from neutral and alkaline soils favourable for keratinolysis. Lower activity was recorded for strains isolated from acidic soils that are less favourable for this process [10].

The main objective of this study was to determine whether quantitative and qualitative diversity is observed in isolated keratinophilic fungi, colonizing soils with different physico-chemical properties. Differences in the mechanical composition (light soils – sandy, heavy soils – clay), the content of humus, nitrogen and phosphorus (more or less rich), pH (soils with acidic, neutral and alkaline pH) were examined when soils where selected for analysis. The quantitative and qualitative composition, species diversity and domination as well as relationships between the frequency of the groups and species dominants and physico-chemical properties of the soils were analyzed to characterize communities of keratinophilic fungi based on morphological criteria.

2. Experimental Procedures

2.1 Soils

Keratinophilic fungi were isolated from four typologically different soils: podzol, cambisol, chernozem and phaeozem. The mechanical composition, macroelement content and pH of the soils are presented in Tables 1 and 2. The determination of the chemical properties of soils were conducted in keeping with methods used in pedologic studies and are given in Table 1 and 2 (humus by the Tiurin method; N-total after sample mineralization using the wet assay method in a mixture of concentrated H_2SO_4 after perhydrol and by flow spectrophotometry; $CaCO_3$ by the Scheiblera method; P_2O_5 by the Egnera-Riehma method, and pH with the potentiometric method).

Soils were sampled once (June 2008) in four arable fields in the Lubelszczyzna region of central-eastern Poland. Between 15 and 20 small samples with a total weight of 2-3 kg were randomly collected from each field. The soil was collected from the topsoil Ap (0-20 cm), placed into plastic bags, and transported to the laboratory. The samples were thoroughly mixed and sifted on a sieve with 2 mm mesh diameter. Soil moisture was *ca*. 60% of the total water content. Soil samples weighing *ca*. 10 grams were placed in Petri dishes, 9 cm in diameter. Fifty dishes (samples) were made from each soil.

2.2 Keratin substrate

Chicken feathers (broilers) obtained from the *Indykpol* poultry plant (Lublin, Poland) were used in the investigations. Feathers were washed, carefully rinsed with distilled water, dried, manually cut into small fragments *ca*. 0.5 cm, and sterilized in an autoclave (121°C, 1 atm., 30 min).

No	Soil type-locality	Mechanical formation	Percentage of fraction of ø in mm											
			>1.0	1.0-0.5	0.5-0.25	0.25-0.1	0.1-0.05	0.05-0.02	0.02-0.006	0.006-0.002	<0.002	1.0-0.1	0.1-0.02	<0.02
1.	Chernozem-Grabowiec	Loess	n.p.	-	-	-	11	47	23	10	7	2	-	40
2.	Phaeozem-Bezek	Medium clay	n.p.	9	20	22	6	7	13	7	16	51	13	36
3.	Podzols-Sobieszyn	Loamy sand	n.p.	6	31	41	7	8.5	1.5	3	2	78	15.5	6.5
4.	Cambisols-Sobieszyn	Heavy clay	n.p.	8	13.5	23.5	10.5	12.5	8.5	7	16.5	45	23	32

Table 1. Grain composition of the soils.

No	Soil type-locality	humus	tot. N %	$CaCO_3$	P_2O_5 mg in 100g of soil	pH in KCl
1.	Chernozem-Grabowiec	3.93	0.270	0.23	13.3	7.15
2.	Phaeozem-Bezek	3.95	0.270	4.67	64.5	7.28
3.	Podzols-Sobieszyn	0.35	0.042	0.00	12.4	3.4
4.	Cambisols-Sobieszyn	1.26	0.098	0.00	16.6	4.4

Table 2. Selected chemical properties of the soils.

2.3 Isolation and identification of keratinophilic fungi based on morphological characters

Keratinophilic fungi were isolated with the hair-baiting method using chicken feathers as the substrate [11]. Plates containing the soil material were sprinkled with sterile feathers and incubated in a humid chamber at 26°C. Mycelial coating was observed after 4-6 weeks and was transferred onto Sabouraud glucose agar with actidione and chloramphenicol [12] Pure cultures were stored in slants with Sabouraud medium (without antibiotics) at +4°C. Macroscopic observations in plates and microscopic observations in microcultures were used to identify the genera and species of the strain material. A research microscope Olympus BX-41 fitted with a digital camera CVIII4 integrated with a computer (Cell-A software for documentation) was used. The final specific classification based on morphological criteria was performed using systematic studies by Domsch *et al*. [13]; van Oorschot [14]; Currah [15].

2.4 Assessment indices of growth of keratinophilic fungi in the soil

The number of soil plates (samples) with fungal growth, the number of genera, species and strains, and the number of species per plate were used to assess the occurrence frequency of keratinophilic fungi. It was also accepted that one soil plate may be colonized only by one strain of a species.

2.5 The assessment of species diversity and fungal prevalence based on morphological criteria

The Simpson index (D) was calculated to analyze the species diversity of keratinophilic fungi using frequencies of individual species:

$$D=1-\sum_{i=1}^{s}(p_i)^2$$

where:

D – index of species diversity, p_i – number of isolates (strains) of the i^{th} species in a particular community of fungi (keratinophilic fungi of individual soils investigated in the study) and the quotient of the number of isolates of this species and the number of all species in this community.

The Simpson index ranges between 0 and 1–1/S, where S is the number of species in a community [16]. The Simpson index uses the theory of probability to measure species diversity of a group of microorganisms.

Species domination (in %) was determined using the formula [17]

$$D=100 \cdot (Sa: S)$$

where

Sa – the total number of isolates of a species

S – the total number of all isolates of a particular group of fungi.

The domination of fungal groups (geophilic dermatophytes, *Chrysosporium*) was determined in the same way, where: Sa – the total number of isolates of a particular group, S – the total number of isolates of all keratinophilic fungi.

The following scale was used to assess the frequency of keratinophilic fungi in the soil [18]: 1% - occurs sporadically; >1-10% - rarely; 11-25% frequently; 26-50% very frequently; >50% in mass.

The correlation index (r) was calculated to determine the relationship between the frequency of species/groups of keratinophilic fungi and soil properties. Arstat software was used for the calculations.

3. Results

3.1 Growth indicators of keratinophilic fungi in the soils

Keratinophilic fungi were recovered from 145 plates (72.5%) of a total of 200 soil samples (plates). The lowest colonization degree by keratinophilic fungi was observed in cambisol (40%). Chernozem and phaeozem were strongly colonized by keratinomycetes, with relevant fungal growth observed in 88% and 90% of soil samples, respectively (Table 3). Non-dermatophytic fungi, known as the *Chrysosporium* group, were recorded quite frequently and colonized 57% of the soil samples. Geophilic dermatophytes colonized 26% of soil samples on average. *Chrysosporium* was most often isolated from chernozem (growth of *Chrysosporium* observed in 88% of soil samples). A low colonization degree by *Chrysosporium* representatives was observed in cambisol and podzol, 24% and 30%, respectively. Geophilic dermatophytes were represented by only one species and the highest colonization degree was observed in podzol (growth of the fungus observed in 60% of soil samples). The colonization degree ranged from 16 to 24% for other soils (Table 3).

A total of 491 fungal strains were isolated from four soil types. 264 strains were classified as keratinophilic fungi. 77% of keratinophilic fungi isolated (203 isolates) were classified as non-dermatophytic species, 33% (61 strains) as geophilic dermatophytes (*T. ajelloi*). The greatest number of isolations of *Chrysosporium* representatives both in total and per plate were recovered from phaeozem and chernozem. A lower frequency of these fungi was recorded in podzol and cabmisol. Reverse quantitative relationships were observed for the population of *T. ajelloi* (Table 3).

The greatest generic and specific richness was observed in the community of keratinophilic fungi in chernozem: five genera and eight species. Three genera and four species were usually recorded in other soils (Table 3).

Soil	Indicator			
	Chernozems form of loess	Phaeozems formed of medium clay	Podzols formed of loamy sand	Cambisols formed of heavy clay
Number of plates colonized:				
total	44	45	36	20
geophilic dermatophytes	8	12	30	11
Chrysosporium	44	45	15	12
Number of:				
genera isolated	5	4	3	3
Species isolates:				
dermatophytes	1	1	1	1
Chrysosporium	7	3	3	3
Number of fungal strains isolated:				
dermatophytes	8	12	30	11
Chrysosporium	82	87	17	17
total	90	99	47	28
Number of species per plate:				
dermatophytes	0.18	0.26	0.83	0.55
Chrysosporium	1.0	1.0	0.41	0.6

Table 3. Indicators of growth of keratinophilic fungi in the soils.

3.2 The species diversity of keratinophilic fungi

The Simpson index was calculated to analyze the species diversity of keratinophilic fungi (Table 4). Both the number and the frequency of individual species were examined. Lower values of the index indicative of a low diversity and considerable prevalence of single species in the community of keratinophilic fungi were recorded for podzol and cambisol. Greater values of the index obtained for phaeozem and especially chernozem show greater diversity and weak species domination within keratinophilic fungi colonizing these soils (Table 4).

3.3 The composition and distribution of species of keratinophilic fungi in the soils

The strain material was identified based on morphological criteria. Seven species of the *Chrysosporium* group were identified. Twenty *Chrysosporium* isolates were determined only to the level of the genus. Five species were imperfect stages (anamorphs), three species were perfect stages (teleomorphs) (Table 5).

Chrysosporium keratinophilum (anamorph) was the most frequently isolated species. Together with the teleomorph *Aphanoascus fulvescens* it constituted 45% of all isolations. *Trichophyton ajelloi* was the second most frequently isolated geophilic dermatophyte (23% of all keratinophilic fungi). A considerable contribution (16.3%) of *Myceliophtora vellerea* (syn. *Chrysosporium asperatum*) within the strain material was observed. These four species represented 84% of the total keratinophilic fungi isolated. The other four species: *Ch. pannicola*, *Ch. tropicum*, *Ch. queenslandicum* and *Ctenomyces serratus*, were classified as occurring sporadically or rarely based on the occurrence frequency (Table 5).

The frequency of individual keratinomycete species was usually dependent on the soil type. The population of *Ch. keratinophilum* and the teleomorph was most numerously represented in phaeozem. Both stages constituted in total 65.5% of all isolations of keratinophilic fungi in phaeozem. They also represented over 50% of total keratinomycetes occurring in cambisol. A smaller contribution of the population of *Ch. keratinophilum* and the teleomorph was also observed for the community of keratinophilic fungi colonizing podzol and chernozem (27-32%).

Soil type				
Chernozem	Phaeozem	Podsol	Cambisol	Total
0.8470	0.7451	0.5356	0.6853	0.8060

Table 4. The Simpson index of species diversity (D) for communities of keratinophilic fungi in the soils.

Species	Soil type				
	Chernozem	Phaeozem	Podzol	Cambisol	Total
	The number of isolated strains				
Chrysosporium group: *Aphanoascus fulvescens* (Cooke) Apinis (teleom.)	5	32	5	5	47
Chrysosporium keratinophilum (Frey) Carmichal (anam. *A. fulvescens*)	19	33	10	10	72
Ch.pannicola van Oorschot & Stalpers (anam.)	10	-	1	-	11
Ch.tropicum Carmichal (anam.)	1	-	-	-	1
Chrysosporium sp. Corda (anam.)	9	9	1	1	20
Ch.queenslandicum Apinis &Rees (anam.)	3	-	-	1	4
Ctenomyces serratus Eidam (teleom.)	5	-	-	-	5
Myceliophtora vellerea van. Oorschot (anam.)	30	13	-	-	43
geophilic dermatophytes: *Trichophyton ajelloi* Ajello (anam.)	8	12	30	11	61

Table 5. Frequency of species of keratinophilic fungi in the soils.

The most numerous population of *T. ajelloi* was observed in podzol. Of 47 keratinomycete strains isolated from podsol, 30 (64%) belonged to *T. ajelloi*. The species (40% of total keratinophilic fungi) was also isolated from cambisol. It occurred less abundantly in chernozem and phaeozem: 10% and 12%, respectively. *Myceliophtora vellerea*, the third most frequently isolated species of keratinophilic fungi, was recorded only in chernozem samples. *M. vellerea* dominated within keratinomycetes in chernozem (Table 5).

3.4 Correlations between the frequency of keratinophilic fungi and some physico-chemical properties of the soils

The correlation index (r) between the frequency of keratinophilic fungi and some edaphic factors was calculated to explain the uneven distribution of individual populations of these fungi in the soils (Table 6).

None of the soil properties we analyzed correlated significantly with the total frequency of keratinophilic fungi. However, correlation indices between ecological factors such as the content of humus, total N and soil pH and the frequency of occurrence of keratinophilic fungi were high (r=0.9). These properties correlated significantly (*) with the total frequency of the representatives of the *Chrysosporium* group. Positive correlation indices show that the total frequency of these fungi in the soil increases together with an increase in the content of humus, nitrogen and soil pH. Positive correlation indices, significant at the level α=0.05, were also obtained for the frequency of occurrence of the most frequently isolated *Chrysosporium* species, *Ch. keratinophilum* with its teleomorph, and the content of $CaCO_3$ and phosphorus (r=0.9841 and 0.9776, respectively). The second most frequent species, *Trichophyton ajelloi*, showed a significant correlation with clay (ø<0.02 mm). The negative correlation index recorded for these two properties shows a reversed relationship between the frequency of *T. ajelloi* and the clay content in the soil. It is also interesting that correlation indices between other edaphic factors (humus, N total, $CaCO_3$, P_2O_5, pH) and the frequency of *T. ajelloi* were also negative; the correlations, however, were not significant.

4. Discussion

Morphological properties of 264 isolates of keratinophilic fungi identified in four typologically different soils revealed significant differences in the occurrence frequency and species composition depending on physical and chemical properties of the soil environment. Conditions recorded in chernozem and phaeozem were favourable for the development and species diversity of keratinophilic fungi. These were: high content of humus, nitrogen and phosphorus, optimum water properties (determined by the humus content and the grain composition), which corresponded to a sufficient supply of oxygen to the soil, good buffer properties caused by the presence of $CaCO_3$, and neutral to slightly alkaline pH.

The observation that keratinophilic fungi preferred soils rich in organic matter and macroelements, with favourable air-water relations, and especially pH≥7.0, was observed by other authors [9,19-22] and was noted in our previous studies Korniłowicz-Kowalska and Bohacz [8]. Of the soil types investigated in these studies, chernozem provided optimum conditions for the development and formation of keratinophilic fungi. We made similar observations in our study: the highest frequency of occurrence as well as the number and the species diversity of the communities of keratinomycetes were recorded in chernozem.

The prevalence of keratinophilic fungi in chernozem and phaeozem is especially closely correlated with soil pH. It was in the optimum range (pH 6.0-9.0) for fungal

Property	Total keratinophilic fungi	Total *Chrysosporium*	*Chrysosporium keratinophilum* + teleomorph	*T.ajelloi*
humus	0.9025	0.9796*	0.7018	-0.7506
Total N	0.8992	0.9782*	0.6977	-0.7556
$CaCO_3$	0.6469	0.5774	0.9841*	-0.2171
P_2O_5	0.6134	0.5576	0.9776*	-0.2561
pH	0.9014	0.9776*	0.7163	-0.7516
Clay (Ø<0.02 mm	0.5245	0.7166	0.4353	-0.9886*

Table 6. Correlation indices (r) between the frequency of keratinophilic fungi and some properties of the soils (significance level α=0.005).

keratinolysis. This overlaps with the optimum values of the activity of keratinolytic proteinases in the majority of species of soil keratinomycetes and is consistent with good conditions (alkalinization) for sulphitosis of native keratin leading to the destruction of disulphide bonds in the protein [23].

As was noticed in previous studies Korniłłowicz-Kowalska and Bohacz [8], "animalization" of chernozem in the past (steppe soils were first inhabited by small mammals) is an additional important ecological factor favourable for a greater species richness of keratinophilic fungi in this soil type in comparison with other soils. Biotope enrichment in native keratin not only stimulates the growth of keratinophilic fungi colonizing them but may also expand the species range of these microorganisms caused by their contamination.

Weaker colonization of the other two soil types, podzol and cambisol, by keratinophilic fungi and in particular the prevalence of single (one or two) populations in these soils, consistent with a lower Simpson index of species diversity, were mostly caused by soil acidification (pH 3.4 and 4.4, respectively).

Some authors [19] did not isolate any keratinophilic fungi from samples of strongly acidic soils (pH_{KCl} from 3.0 to 4.5). Our previous research into strongly acidic soils (pH_{KCl} 3.36; 4.06; 4.19; 4.29) indicates that populations of one species have been selected [8]. As well strong acidification, other properties unfavourable for keratinophilic fungi observed in the podzol we examined included low fertility, high permeability (light sandy soil) and the negative water balance related to it. A high content of clay (heavy clay soil) was an additional factor recorded in cambisol and a sufficient amount of air in the soil was not ensured despite an improvement in water capacity.

Research into the species structure of communities of soil keratinomycetes shows the prevalence of *Chrysosporium* populations in soils with pH≥7.0 [7,8,24]. According to the ecological classification of keratinophilic fungi proposed by Hubálek [25], keratinolytic species of *Chrysosporium* are mostly neutrophilic and alkalinophilic, and keratinolytic species of geophilic dermatophytes are mostly acidophilic and neutrophilic as the optimum pH values of the keratinolytic activity of *Chrysosporium* are usually higher than those of geophilic dermatophytes [7,25].

The morphological identification of the strain material isolated in our study confirmed the domination of representatives of *Chrysosporium* and geophilic dermatophytes in soils with different pH. Geophilic dermatophytes were represented by one species: *Trichophyton ajelloi*, classified as an acidophilic fungus by Hubálek [25]. Its populations have the greatest frequency in very acidic soils (podzol). A statistically confirmed growth stimulation of *T. ajelloi* in acidic and strongly acidic soil was previously recorded by Korniłłowicz-Kowalska and Bohacz [8]. We observed a similar relationship here but the correlation (r=-0.7516) was not significant. However, a significant negative correlation was recorded between the frequency of occurrence of *T. ajelloi* and clay (r=-0.9886, significance level α=0.05). Both figures indicate that *T. ajelloi* is prevalent in acidic soils, with lower fertility, excessively permeable (light soils). Reverse tendencies in the population distribution were observed in the *Chrysosporium* group. The frequency of *Chrysosporium* representatives increased as pH increased (r=0.9777, α=0.05). It was also positively correlated with the content of humus and nitrogen, which indicates their preference for more fertile soils. This was reflected in the occurrence frequency of species such as *Chrysosporium keratinophilum* together with its teleomorph *Aphanoascus fulvescens*, *Ch. pannicola*, *Myceliophtora vellerea* and *Ctenomyces serratus*. The prevalence of these species in environments with pH≥7.0 was also observed previously [8,24,26]. A positive correlation between the frequency of *Ch. keratinophilum* together with its teleomorph and the values of $CaCO_3$ and phosphorus was also observed. As an increase in their content in the soil is accompanied by an increase in pH, the effect was caused by the selective influence of pH on the frequency rather than a direct impact of $CaCO_3$ and phosphorus on these fungi.

The uneven distribution of keratinophilic fungi at the level of population in soils with different physico-chemical properties may be caused by the differences in their physiological properties and, in consequence, tolerance to changes in ecological factors. Physiological variation of strains of the same species of different origin was reported in a study by Korniłłowicz-Kowalska [2,10]. Our study shows that of the species dominants we isolated, greater tolerance to changes in soil pH was recorded for *Ch. keratinophilum*, classified together with its teleomorph as an alkalotolerant fungus growing in the pH range between 4.5 and 9.5 [7]. *T. ajelloi* is considered to be exceptionally acidophilic, and is sporadically recorded in soils with pH>6.0 [27]. However, our studies show that *T. ajelloi* was frequent or very frequent in acidic soils, and it occurred relatively frequently in soils with neutral to alkaline pH. This shows intraspecific heterogeneity of *T. ajelloi* populations. Results of molecular identification showed that the *T. ajelloi* population had greater genetic variation, which is related to the origin of the strains (unpublished data).

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