CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 63 (4-5) 433-439, 2014.
UDC: 63(497.1)(051)-„540.2“ ISSN: 0350-1205

UDC: 636.5:658.2

ISOLATION OF KERATINOLYTIC FUNGI FROM SOIL SAMPLES OVERCAST BY PRESENCE OF CHICKEN

JAROSLAVA KAČINOVÁ, DANA TANČINOVÁ[1]

SUMMARY: Keratinophilic fungi are an important group of fungi that occurred in soil. The aim of this study was to isolate and identify keratinolytic fungi from the 10 soil samples overcast by presence of chicken from Slovakia. Isolation of the fungi was performed by hair bait technique. The isolated colonies were identified by morphologic feature of macro- and microconidia. The isolated keratinolylic fungi were classified into 10 species belonging to 6 genera. From the total of 123 isolates of keratinolytic fungi represents Chrysosporium keratinophilum 39 (31.7%), Trichophyton ajelloi (30.1%), Myceliophthora vellerea (10.6%), Trichophyton terrestre and Chrysosporium evolceanui (6.5%), Chrysosporium fluviale (5.7%), Microsporum gypseum (4.1%), Arthroderma uncinatum (2.4%), Purpureocillium lilacinum (1.6%), Chrysosporium indicum (0.8%). Most of the fungi (96 isolates) were isolated from the soils with the pH range of 7 to 8.

***Keywords*:** keratinolytic fungi, free-range chickens, *Chrysosporium, Trichophyton.*

INTRODUCTION

Keratins are valuable but unavailable fibrous animal proteins. They are components of a range of by-products occurring especially abundantly in slaughterhouses and meat and poultry plants: skin remains, bristle, animal hair, horns and hooves, feathers etc. (Korniłłowicz-Kowalska and Bohacz, 2011). Keratins, which are among the hardest-to-degrade animal proteins, are the major component proteins in poultry feathers and are characterized by a tightly packed form in α- helixes and β sheets with a high degree of disulphide bonds (Yasushi et al., 2009). Keratinolytic fungi specialize in the decomposition ofkeratin, being the main component of keratinous substrata. Keratinophilic fungi associate keratinolytic fungi, utilizing non-protein components of the substrata or the products of keratin decomposition (Ulfig, 2005). Soil is rich in keratinous components

Original scientific paper / *Originalni naučni rad*

[1]Jaroslava Kačinová, MSc, PhD., professor, Dana Tančinová, MSc, PhD, Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Nitra, Slovak Republic.
Corresponding author: Dana Tančinová, e-mail: dana.tancinova@uniag.sk, phone: +421 37 641 4433.

which are most conducive for the growth and occurrence of keratinophilic fungi (Jain and Sharma, 2012). Rich keratinous materials in soil are the most reason of high incidence of keratinophilic fungi (Zarrin and Haghgoo, 2011). The prevalence of these fungi depends on different factors, such as the presence of creatinine in the soil, pH, and geographical location (Deshmukh and Verekar, 2006). The process of keratin decomposition has also been found to be very fast in soil and it plays a very important role in energy transformation and nutrient cycling in soil (Kushwaha, 2000).

The aim of this study was to isolate and identify keratinolytic fungi from the soil samples overcast by occurrence of chicken.

MATERIAL AND METHODS

Isolation of keratinolytic fungi from soils overcast by occurrence of chicken: A total of 10 samples were taken from different location from Slovakia (Table 1). Keratinophilic fungi were isolated by the hair-baiting method (Vanbreuseghem, 1952). For the isolation of keratinophilic fungi, only the superficial layer 5 cm of the humus horizon was used. Soil samples were poured into Petri dish (up to 10 subsample), 1 sample = 10 subsamples. Based on soil moisture, we applied 10 mL cykloheximid 500 mg/L + 50 mg/L chloramphenicol solutions. As bait were applied 10 fragments of sterilized horsehair on a Petri dish. Cultivation was carried out at 25±1°C for 2 – 3 months (every week check if any growth does occur on the fragments). The pH of each soil sample (25 g) was measured using a pH meter (Sentron), after dilution in sterile distilled water 125 ml with 10 minutes of agitation.

Table 1. Overview of studied soil samples

Soil samples	Locality	pH soil
1	Trnava 1	6.4
2	Trnava 2	8.1
3	Nitra 1	8.4
4	Nitra 2	6.7
5	Smolenice	7.5
6	Pezinok	8.6
7	Nové Zámky	6.4
8	Michalovce 1	7.7
9	Michalovce 2	7.1
10	Michalovce 3	7.7

Isolation of the keratinolytic fungi from colonized hair fragments:Potato Dextrose Agar (PDA) (Conda, Spain) was prepared and 100 µL of the antibiotic solution (chlortetracycline/chloramphenicol) was applied on the surface and evenly spread. Solution (chlortetracycline/chloramphenicol) was prepared: 100 mg/L + 100 mg/L

osmotic water, sterilization at 120°C for 15 min. The mycelium was transferred from a fragment colonized hair into the PDA plate with antibiotic solution. We did three / four lines on plate (scars) to be sure that the contaminating fungi will be well separated from the keratinophillic fungi. The cultivation temperature for PDA plates was 25±1°C, darkness, 4 – 6 days, until colonies appeared. Cultures were transferred to 2% Sabouraud Glucose Agar (SGA) (Conda, Spain) for identification. These plates were incubated at 25±1°C for 7 days and then were used for identification.

Identification of keratinolytic fungi:The identification of the resulting keratinophilic and keratinolytic fungi was based on their phenotypic characteristics according to De Hoog et al. (2000), Domsch et al. (1980) and Van Oorschot (1980).

RESULTS AND DISCUSSION

All off tested samples were positive on the keratinolylic fungi. Keratinolytic fungi belong to 10 species representing 6 different genera (Table 2). A total diversity of keratinolytic fungi recovered in this study accounted 123 isolates. The most common isolates were *Chrysosporium keratinophilum* (31.7 %), *Trichophyton ajelloi* (30.1 %) and *Myceliophthora vellerea*(10.6 %).

Table 2. Frequency of keratinolytic fungi isolated from soil samples in free-range chicken

Species	Isolates	
	n	%
Arthroderma uncinatum	3	2.4
Chrysosporium evolceanui	8	6.5
Chrysosporium fluviale	7	5.7
Chrysosporium indicum	1	0.8
Chrysosporium keratinophilum	39	31.7
Microsporum gypseum	5	4.1
Myceliophthora vellerea	13	10.6
Purpureocillium lilacinum	2	1.6
Trichophyton ajelloi	37	30.1
Trichophyton terrestre	8	6.5
Total	123	100

From the total 123 isolates of keratinophilic fungi represents *Chrysosporium keratinophilum*(39 isolates), which represent 31.7 %. Most *Chrysosporium* species are keratinophilic fungi, living on remains of hairs and feathers in soil (De Hoog et al., 2000). *Chrysosporium keratinophilum* and *Chrysosporium queenslandicum* are geophilic keratinolytic species (BSL-1). *Chrysosporium keratinophilum* was repeatedly isolated from onychomycoses and superficial infections and *Chrysosporium queenslandicum* was isolated from skin and nail infections (Reboux et al., 2005).*Chrysosporium* species are the most common keratinophilic fungi isolated from soil in many parts of the world (Shadzi et al., 2002; Deshmukh, 2002; Papini, 1998). Labuda et al. (2008) isolated (*Chrysosporium europae, Chrysosporium fluviale* and *Chrysoporiumminutisporosum*) from the soil and children's sandpit samples in city park of Nitra and Nová Baňa (Slovakia).*Chrysosporium synchronum*was isolated by Labuda et al. (2009) as rediscovered in Slovakia.Microscopic fungi, namely *Chrysosporium zonatum*, *Malbranchea cinnamomea* and *Myceliophthora thermophila* were also isolated by Labuda et Kačinová (2007) from compost and sludge samples from two different locations in Slovakia.

*Trichophyton ajelloi*which represent 37 isolates (30.1%)is a geophilic fungus with a worldwide distribution. It cause infections in human and animals, but they are not often reported.

Microsporum gypseum which represent 5 isolates (4.1%) is geophilic fungus. This species is worldwide in distribution. The fungus also infects a variety of animals. The species produces *tinea corpris* and *tinea capitis* (Howard, 2003). *Microsporum gypseum* was isolated from park soils in Gorgan, north of Iran (Malek et al., 2013) and this species was more frequent than other keratinophilic fungi (22.96%).

Two species,*M. gypseum* and *T. ajelloi*, seem to possess the highest potential to digest keratinous materials regardless of the type of substrate (Blyskal, 2009).Many factors impact the degree of material biodeterioration, including microbial metabolic products, i.e., enzymes, acids, and pigments; chemical composition of the material and whether or not additional substances, such as dyes, are contained in the materials; moisture content and its accessibility to microorganisms; history of the material and its age; and local microclimate: availability of oxygen and light, temperature, and relative humidity (Blyskal, 2009).

Table 3. Keratinolytic fungi isolated from soil samples with different pH

Fungal genus	pH		
	6 – 7 (n)	7 – 8 (n)	8 – 9 (n)
Arthroderma	0	3	0
Chrysosporium	0	46	9
Microsporum	0	5	0
Myceliophthora	3	10	0
Purpureocillium	0	2	0
Trichophyton	12	30	3
Total	15	96	12

n= number isolates.

In this study, all 123 keratinolytic fungi were isolated from the soils with pH between 6 and 9 (Table 3). In the present study the *Chrysosporium* spp. (46isolates) and *Trichophyton* spp. (30isolates) were mostly detected in the soil samples with pH range of 7 to 8. We found 12 strains *Trichophyton* spp. from the soil samples with pH of 6 to 7. Asahi et al. (1985) demonstrated that keratinolytic enzymes were produced in pH of 6 to 9, and particularly the extracellular keratinase was active in pH = 9. The species *Arthroderma uncinatum*, *Microsporum gypseum* and *Purpureocillium lilacinum* were isolated from only the soil samples with pH of 7 to 8. Da Silvia Pontes and Oliveira (2008) also recorded that the keratinophilic fungi develop much better in alkaline pH. According to a research conducted by Jain and Sharma (2011), most of the keratinophilic fungi were isolated from pH 6.5 to 8.5.

CONCLUSION

This research reports the prevalence and distribution of keratinolytic fungi in soil samples in free-range chicken (*Gallus domesticus*) from the Slovakia. Keratinolytic activity has an important role in degradation of feather in natural environment.

ACKNOWLEDGMENTS

The research leading to these results has received funding from the European Community under project no 26220220180: Building Research Centre „Agrobiotech".

REFERENCES

ASAHI,M., LINDQUIST, R., FUKUYAMA, K.: Purification and characterization of major extracellular proteinases from *Trichophyton rubrum*. Biochemical Journal, 232(1)139– 144, 1985.

BLYSKAL, B.: Fungi utilizing keratinous substrates. International Biodeterioration and Biodegradation, 63:631–653, 2009.

DA SILVIA PONTES, Z.B.V., OLIVEIRA, A.C.: Dermatophytes from urban soilsbvin João Pessoa, Paraíba, Brazil. Revista Argentina de Microbiologia, 40:161–163, 2008.

DE HOOG, G. S. , GUARRO, J. – GENÉ, J. – FIGUERAS, M. J.: Atlas of Clinical Fungi, 2. vyd. Utrecht : Centraalbureau voor Schimmelcultures. p. 383 – 393. ISBN 90-70351-43-9, 2000.

DESHMUKH, S.K., VEREKAR, S.A.: The occurrence of dermatophytes and other keratinophilic fungi from the soils of Himachal Pradesh (India). Czech Mycology, 58(1-2)117–122, 2006.

DESHMUKH, S.K.: Incidence of keratinophilicfungi from selected soils of Kerala state (India). Mycopathologia, 156:177-181, 2002.

DOMSCH, K.H. ,GAMS, W., ANDERSON, T.H.: Compendium of soil fungi. London: Academic press, 1980, 859 p., ISBN 0122204018, 1980.

HOWARD, D.H.: Pathogenic Fungi in Humans and Animals. Marcel Dekker, New York, 2003.
JAIN N, SHARMA M.: Distribution of dermatophytes and other re-lated fungi in Jaipur city, with particular reference to soil pH. Mycoses, 54(1)52–8, 2011.
JAIN, N., SHARMA, M.: Biodiversity of keratinophilic fungal flora in university campus, jaipur, India. Iran J Public Health, 41(11)27–33, 2012.
KORNIŁŁOWICZ-KOWALSKA, T., BOHACZ, J.: Biodegradation of keratin waste: Theory and practical aspects. Waste Management, 31:1689–1701, 2011.
KUSHVAHA, R.K.S.: The genus *Chrysosporium*, its physiology and biotechnological potenctial. In Kushwaha, R. K. S. & Guarro, J. (eds), Biology of dermatophytes and other keratinophilic fungi, Bilbao. Revista Iberoamericana de Micologia, p. 66 – 92. ISBN 84-607-0711-3, 2000.
LABUDA, R., KAČINOVÁ, J.: New records of microscopic fungi from the genus *Chrysosporium*, *Malbranchea* and Myceliophthora from Slovakia. Micromyco 2007: Proceedings of the workshop MICROMYCO 2007 held in České Budějovice, Czech Republic, September 4. – 5., Institute of Soil Biology Biology Centre, Academy of Sciences of the Czech Republic, p. 52 – 59. ISBN 978-80-86525-10-5, 2007.
LABUDA, R., KEČKÉŠOVÁ, L., GUARRO, J., CANO, J.: *Chrysosporium synchronum* rediscovered in Slovakia. Biologia 64/5: 890—892, Section Botany DOI: 10.2478/s11756-009-0173-9, 2009.
LABUDA, R., NAĎOVÁ, L., VÉN, T.: First record of *Chrysosporium europae*, *C. fluviale* and *C. minutisporosum* in Slovakia. Biologia, 63:38–39, 2008.
MALEK, M., MOOSAZADEH, M., HANAFI, P., NEJAT, Z.A., AMINI, A., MOHAMMADI, R., KOHSAR, F., NIKNEJAD, F.: Isolation of Keratinophilic Fungi and Aerobic Actinomycetes From Park Soils in Gorgan, North of Iran. Jundishapur J Microbiol. December; 6(10): e11250. DOI: 10.5812/jjm.11250, 2013.
PAPINI, R., MANCIANTI, F., GRASSOTTI, G., CARDINI ,G.: Survey of keratinophilic fungi isolated from city park soils of Pisa, Italy. Mycopathologia, 143:17-123, 1998.
REBOUX, G., COMPAROT, S., KIRCHGESNER, V., BARALE, T.: A propos de 19 souches de *Chrysosporium* isolées au Centre Hospitalier Universitaire de Besancon. Bilan de 10 années: 1984-1994. Medical Mycology, 5:105–110, 2005.
SHADZI, S., CHADEGANIPOUR, M., ALIMORADI, M.: Isolation of keratinophilic fungi from elementary schools and public parks in Isfahan, Iran. Mycoses, 45:496-499, 2002.
ULFIG, K.: Sludge Liming Decreases the Growth of Keratinolytic and Keratinophilic Fungi. Polish J. Environ. Stud., 15(2)341-346, 2005.
VAN OORSCHOT, C.A.N.: A revision of *Chrysosporium* and allied genera. Studies in Mycology, vol. 20, 1980, p. 1 – 89, ISSN 0166-0616, 1980.
VANBREUSEGHEM, R.: Technique biologique pour I'isolement des dermatophytes dusol," Annales de la Soci´et´e Belge deM´edecine Tropicale, vol. 32, pp. 173–178, 1952.
YASUSHI, S., TATSUNOBUI, M., KUNIHIKO, W.: Decomposition of intact chicken feathers by a thermophile in combination with an acidulocomposting garbage-Treatment process. Bioscience Biotechnology Biochemistry, 73(11)2519-2521, 2009.
ZARRIN M, HAGHGOO R. Survey of keratinophilic fungi from soils in Ahvaz, Iran. Jundishapur J Microbiol., 4(3)191-4, 2011.

IZOLACIJA KERATINOLITIČKIH GLJVA IZ UZORAKA ZEMLJIŠTA NA KOME SU BORAVILI PILIĆI

JAROSLAVA KAČINOVÁ, DANA TANČINOVÁ

Izvod

Keratinolitičke gljive su važna grupa gljiva, prisutna u zemljištu. Cilj ovog rada je bio da se izoluju i identifikuju keratinolitičke gljive iz 10 uzoraka zemljišta, na kome su boravili pilići u Slovačkoj. Na izolovanim kolonijama su identifikovana morfološke osobine makro i mikrokonidija. Izolovano je 10 vrsta i 6 rodova keratinolitičkih gljivica. Od ukupno 123 izolata, *Chrisosporium keratinophilum* je zastupljen sa 31.7%, *Trichophiton ajelloi* sa 30.1%, *Miceliophthora vellerea* sa 0.6%, *Trichophiton Terrestre* i *Chrisosporium evolceanui* sa 6.5%, *Chrisosporium Fluviale* sa 5.7%, *Microsporum gipseum* sa 4.1%, *Arthroderma uncinatum* sa 2.4%, *Purpureocillium lilacinum* sa 1.6% , a *Chrisosporium indicum* sa 0.8%. Većina gljivica je izolovana iz zemljišta u kome je vrednost pH iznosila 7 ili 8.

Ključne reči: keratinolitičke gljive, slobodno držanje, pilići, *Chrysosporium, Trichophyton.*

Received / *Primljen*: 03.11.2014.
Accepted / *Prihvaćen*: 17.11.2014.