

IDENTIFICATION OF A MOULD OF THE *SCEDOSPORIUM* GENUS ISOLATED FROM MILK OF A COW WITH MASTITIS

Dubravka MILANOV¹, Oliver STEVANOVIĆ², Milan ĐILAS³,
Nevenka ALEKSIĆ⁴

¹Novi Sad Scientific Veterinary Institute, Novi Sad, Serbia

²Dr Vaso Butozan Veterinary Institute of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina

³Institute of Public Health of Vojvodina, Novi Sad, Serbia

⁴University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia

Received 19 February 2025; Accepted 05 July 2025

Published online: 08 July 2025

Copyright © 2025 Milanov et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

How to cite: Dubravka Milanov, Oliver Stevanović, Milan Đilas, Nevenka Aleksić. Identification of a mould of the *Scedosporium* genus isolated from milk of a cow with mastitis. Veterinarski Glasnik, 2025. 79(2): 148-155. <https://doi.org/10.2298/VETGL250219009M>

Abstract

This case report presents the basic parameters that led to the identification of a mould from the genus *Scedosporium*, isolated from mastitic cow's milk. The macroscopic and microscopic characteristics of the isolate and the result of the identification by MALDI-TOF mass spectrometry are given. As this is the first report on the identification of *Scedosporium* in the Balkan region, the data could be useful for laboratories in the region working with materials of animal origin.

Keywords: cow, mastitis, mould, *Scedosporium*

CASE PRESENTATION

The Scientific Veterinary Institute in Novi Sad was presented with an isolate from milk obtained from a dairy cow with clinical mastitis in one of the udder quarters. The cow was from the vicinity of Lopare, Republic of Srpska, Bosnia and Herzegovina. The cultural and microscopic features of the isolate were characteristic of a mould. Still, the genus remained unknown; in long-standing practice, such a mould had not been isolated either from milk obtained from cows with mastitis or from any other animal-

*Corresponding author – e-mail: nenaaleksic@vet.bg.ac.rs

derived material. At first, the milk sample was suspected to have been contaminated with mould(s) from the environment. Therefore, a veterinary surgeon repeated the sampling from the infected udder under aseptic conditions. The cultivation resulted in the isolation of what looked to be the same species in pure culture. The veterinary surgeon who took the milk samples pointed to the poor hygienic conditions in the housing, mould-infested bedding and the previously unsuccessful antibiotic treatment of the udder.

Macroscopic characteristics of the isolate

The isolate was subcultivated on standard laboratory culture media: blood agar, Sabouraud dextrose agar, Dichloran Glycerol (DG 18) agar (all Biokar Diagnostics) and liquid thioglycollate medium. The plates were incubated in both aerobic and anaerobic conditions (GasPak EZ, Becton Dickinson, U.S.) at 25 °C and 37 °C. In subculture, growth of the mould was noted after 24 hours' incubation in both aerobic and anaerobic conditions at 25 °C and 37 °C on blood agar and Sabouraud dextrose agar. There was no growth on the DG18 agar. The characteristics of the isolate growth at different ages on different media are presented in Figures 1, 2, 3 (solid media) and Fig. 4 (liquid medium).

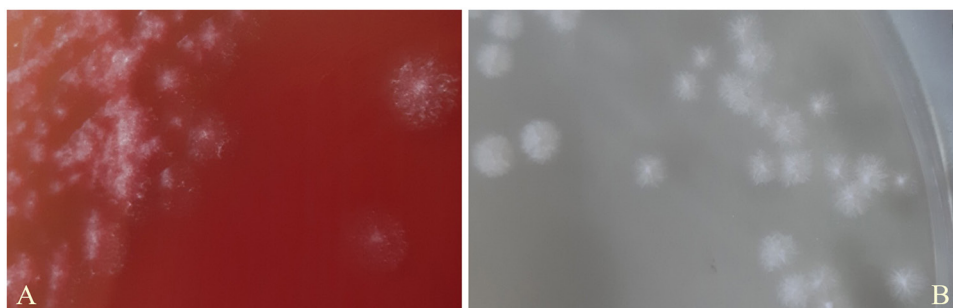


Figure 1. Colonies after 24 hours' incubation in aerobic conditions and 37 °C on blood agar (A) and Sabouraud dextrose agar (B). The colonies were tiny, white and cotton-like, with cobweb-like surfaces, compact centres and irregular edges.



Figure 2. Colonies after five days' incubation in aerobic conditions on Sabouraud dextrose agar at 37 °C. The colonies were of distinct form, cotton-like, convex, sagging in the periphery, greyish white in colour, with whiteish edges (A). It was possible to detach the surface mycelia

with an inoculating loop, but the compact centres remained tightly attached to the culture medium. On the underside of the plates, a yellow pigment and brownish centres of the colonies were notable (**B**).

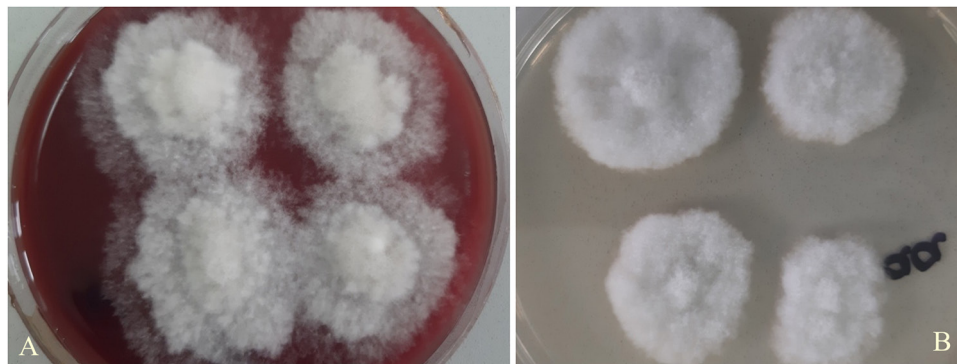


Figure 3. Colonies after five days' incubation under anaerobic conditions at 37 °C on blood agar (**A**) and Sabouraud dextrose agar (**B**). The colonies were large, white, with compact centres, cobweb-like surfaces and without pigmentation.

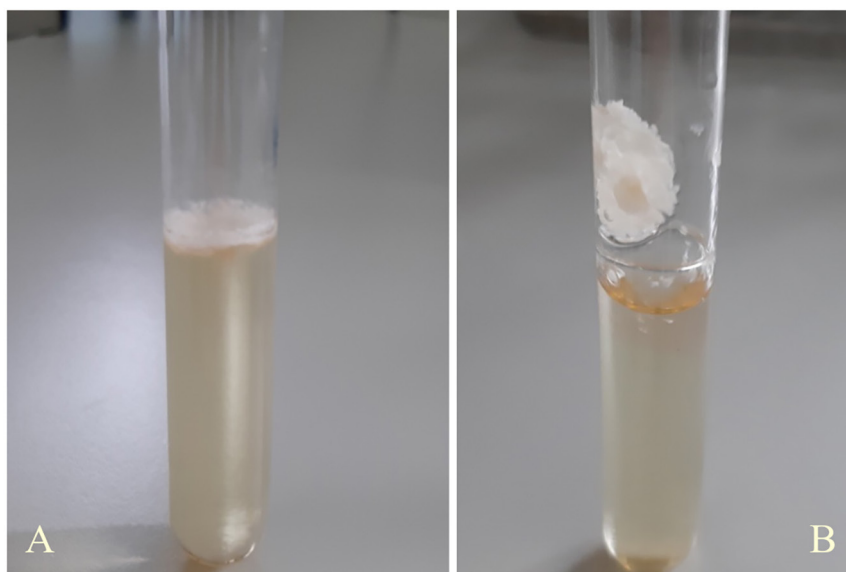


Figure 4. Growth of the isolate in liquid thioglycollate medium after five days' incubation at 37 °C. Mycelial growth at the liquid-air interphase (**A**). The compact structure was completely detachable from the liquid with an inoculation loop (**B**).

Microscopic features

Microscopic examination revealed hyphae and ovoid-shaped conidia (Fig. 5).

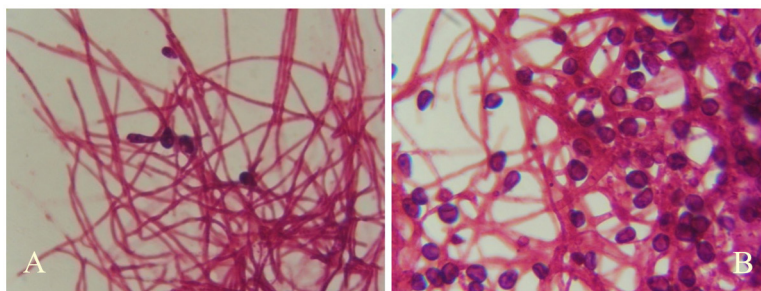


Figure 5. Microscopic features of the mould isolate (Gram-stain), after five days' incubation (magnification 1000×). Hyphae and ovoid-shaped conidia (**A** and **B**).

Based on the culture characteristics and microscopic morphology of the fungus, its genus remained unknown, so help was requested from the Institute of Public Health of Vojvodina, Serbia, with which multi-year cooperation in the identification of pathogen species in human and veterinary medicine has been fruitful.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

The Institute of Public Health of Vojvodina used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), (Bruker Daltonics, Germany), and applied the MSP Library, Filamentous Fungi. The isolate was then identified as *Scedosporium* sp., score value 2.03 (Fig. 6).

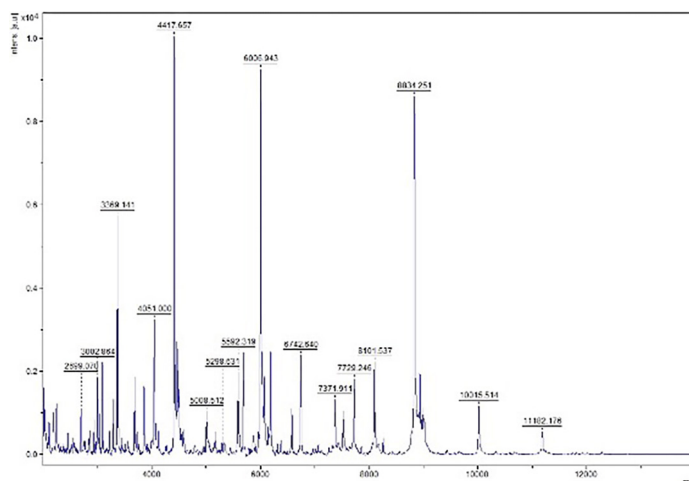


Figure 6. Spectrum of the *Scedosporium* isolate generated by MALDI-TOF Bruker flexControl software (Bruker Daltonics GmbH & Co., Germany).

Scedosporium spp. are ascomycetous fungi belong to the genus *Scedosporium*, family Microascaceae, order Microascales, and phylum Ascomycota.

COMMENT

Scedosporium (former *Pseudallescheria*, which was the name for the teleomorph) is a genus of filamentous fungus of the *Microascales* order and *Microascaceae* family (Elad, 2011). The species of the genus are generally considered to be saprophytes, ubiquitous in soil and in standing and polluted waters, mainly in human-impacted environments (Cortez et al., 2008; Carnovale et al., 2022; Mello et al., 2022). They have been isolated from domestic and wild animal faeces, cattle gut microbiota (Wen et al., 2018) and manure (Elad, 2011).

The genus currently comprises ten recognized species: *S. aurantiacum*, *S. cereisporum*, *S. desertorum*, *S. deboogii* and *S. minutisporum*, and *S. apiospermum* species complex which includes *S. angustum*, *S. apiospermum*, *S. boydii*, *S. ellipsoideum*, and *S. fusoidium*. These species can be distinguished phylogenetically by comparing the sequences of a fragment of the β -tubulin gene (TUB2) (Abrantes et al., 2021; Mello et al., 2022). Among them, *S. apiospermum*, *S. boydii* and *S. aurantiacum* are clinically relevant and the most pathogenic species for human and animals (Chen et al., 2021).

The high degrees of intrinsic antifungal resistance render these infections difficult to treat successfully (Lackner et al., 2012). In healthy individuals, the respiratory organs are primarily endangered (the lungs, ears and sinuses), resulting from the inhalation or traumatic inoculation of *Scedosporium* conidia (Ramirez-Garcia et al., 2018; Carnovale et al., 2022; Mello et al., 2022).

In veterinary medicine, various infections of domestic animals caused by *Scedosporium*/*Pseudallescheria* moulds have been described: inflammation of the reproductive tract, mycetoma, keratitis, upper respiratory tract infections, and onychomycosis in horses; abortions, infections of the respiratory organs and mastitis in ruminants; abortion in pigs; dermatitis, disseminated infections, osteomyelitis, and infections of the kidneys, heart, blood vessels, lymph nodes, brain and eyes in dogs (Thompson et al., 1978; Smedes et al., 1992; Elad, 2011; di Teodoro et al., 2020; Tsoi et al., 2021). Although the prevalence of infections in animals seems to be lower than that in humans, the most probable reason is that the majority of cases remain undiagnosed or misdiagnosed (di Teodoro et al., 2020). The sporadic incidence of fungal mastitis in dairy cows could result from infective agents that enter through udder injuries caused by milking machines. Extensive and indiscriminate use of antibiotics for treating mastitis of other aetiology could certainly contribute to fungus-caused mastitis (Wen et al., 2018).

In resource-limited settings, culture and phenotypic approaches are pivotal to diagnostics (Chen et al., 2021). Identification of *Scedosporium* genus based exclusively on culture morphology is disputable (Tsoi et al., 2021), so MALDI-TOF/MS is necessary (Chen et al., 2021; Tsoi et al., 2021). In spite of that, similarities between microorganisms

and the number of spectra of known species/genera in the corresponding database used by the MALDI-TOF MS could result in low scores and limit the accurate determination of any given sample. However, species identification is possible with molecular methods only (Elad, 2011; Luna-Rodriguez et al., 2019; Tsoi et al., 2021). In fact, nucleotide-sequence-based analysis appears to be the gold standard for the identification of *Scedosporium* moulds (Carnovale et al., 2022).

“There appears to be a lower incidence of infections with SPCF [*Scedosporium*/*Pseudallescheria* complex fungi] in veterinary species, although this may be related to a lack of awareness of these diseases” (Elad, 2001). The first identification of an animal-derived isolate in the Balkan region was successful owing to cooperation with the Institute of Public Health of Vojvodina, where MALDI-TOF mass spectrometry analysis was used. Infections caused by *Scedosporium* spp. are linked to the irrational administration of antibiotics and corticosteroids and the pollution of the environment by human activities, which we have been witnessing. Thus, information on the presented case could benefit the regional veterinary laboratories.

Ethical statement

Ethical approval was not required for this study as it did not involve any experimental intervention, treatment, or procedures on animals beyond routine clinical examinations and milk sample collection performed for diagnostic purposes.

Acknowledgements

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia through contract nos. 451-03-136/2025-03/200143 and 451-03-136/2025-03/200031.


Authors' contributions


DM and OS completed the classical microbiological examinations and wrote this paper draft, MĐ did the MALDI-TOFF MS, and NA edited and critically reviewed the work. All the authors have read and approved the final version of the manuscript.


Competing interests


The authors declare no competing interests pertinent to the present work.

ORCID iDs

Dubravka Milanov  <https://orcid.org/0000-0003-1987-8211>

Oliver Stevanović  <https://orcid.org/0000-0002-1548-9748>

Milan Đilas  <https://orcid.org/0000-0001-7772-4355>

Nevenka Aleksić  <https://orcid.org/0000-0003-2093-8656>

REFERENCES

- Abrantes R.A., Refojo N., Hevia A.I., Fernández J., Isla G., Córdoba S., Dávalos M.F., Lubovich S., Maldonado I., Davel G.O., Stehigel A.M. 2021. *Scedosporium* spp. from Clinical Setting in Argentina, with the Proposal of the New Pathogenic Species *Scedosporium americanum*. *Journal of Fungi* (Basel). 7(3):160. doi: 10.3390/jof7030160
- Carnovale S., Epelbaum C., Abrantes R., Cordobac S., Cabrera C., Caracciolo B. 2022. *Scedosporium aurantiacum*. First isolation in Argentina from a previously healthy patient after traumatic inoculation. *Revista Argentina de Microbiología*, 54, 318-321. <https://www.sciencedirect.com/science/article/pii/S0325754122000189>
- Chen S.C.-A., Halliday C.L., Hoenigl M., Cornely O.A., Meyer W. 2021. *Scedosporium* and *Lomentospora* infections: Contemporary microbiological tools for the diagnosis of invasive disease. *Journal of Fungi*, 7, 23.
<https://doi.org/10.3390/jof7010023>
- de Hoog, G.S., Marvin-Sikkema, F.D., Lahpoor, G.A., Gottschall, J.C., Prins, R.A., Guého, E. 1994. Ecology and physiology of the emerging opportunistic fungi *Pseudallescheria boydii* and *Scedosporium prolificans*. *Mycoses*, 37(3-4), 71–78. <https://pubmed.ncbi.nlm.nih.gov/7845423/>
- di Teodoro G., Averaimo D., Primavera M., Santoleri D., Giovannini G., Cocco A., Di Francesco G., Malatesta D., Defourny S., D'Alterio N., Curini V., Di Domenico M. Petrini A. 2020. Disseminated *Scedosporium apiospermum* infection in a Maremmano-Abruzzese sheepdog. *BMC Veterinary Research*, 16:372. <https://doi.org/10.1186/s12917-020-02597-9>
- Elad D. 2011. Infections caused by fungi of the *Scedosporium/Pseudallescheria* complex in veterinary species. *The Veterinary Journal*, 187 (1): 33–41. <https://pubmed.ncbi.nlm.nih.gov/20580291/>
- Lackner M., de Hoog G.S., Verweij P.E., Najafzadeh M.J., Curfs-Breuker I., Klaassen C.H., Meis J.F. 2012. Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. *Antimicrobial Agents and Chemotherapy*, 56(5):2635–2642. <https://pubmed.ncbi.nlm.nih.gov/22290955/>
- Lackner M., de Hoog G.S., Yang L., Ferreira Moreno L., Ahmed S.A., Andreas F., ... Liporagi Lopes L.C. 2014. Proposed nomenclature for *Pseudallescheria*, *Scedosporium* and related genera. *Fungal Diversity*, 67: 1-10.
https://www.academia.edu/33384253/Proposed_nomenclature_for_Pseudallescheria_Scedosporium_and_related_genera
- Lagneau P.E., Bierny M., Vercouter M. 2000. Isolation of *Scedosporium apiospermum*/*Pseudallescheria boydii* from a bovine mastitis case. *Journal de Mycologie Medicale*, 10(1): 43-45.
<https://www.cabidigitallibrary.org/doi/full/10.5555/20001202170>
- Luna-Rodriguez C.E., Trevino-Rangel R.J., Montoya A.M., Becerril-García M.A., Andrade A., Gonzalez G.M. 2019. *Scedosporium* spp.: Chronicle of an emerging pathogen. *Medicina Universitaria*, 21(1): 4-13.
https://www.academia.edu/89133456/Scedosporium_spp_Chronicle_of_an_emerging_pathogen
- Mello T.P., Barcellos I.C., Aor A.C., Branquinha M.H., Santos A.L.S. 2022. Extracellularly released molecules by the multidrug-resistant fungal pathogens belonging to the *Scedosporium* genus: An overview focused on their ecological significance and pathogenic relevance. *Journal of Fungi*, 8(11): 1172. <https://pubmed.ncbi.nlm.nih.gov/36354939/>

- Ramirez-Garcia A., Pellon A., Rementeria A., Buldain I., Barreto-Bergter E., Rollin-Pinheiro R. Hernando F.L. 2018. *Scedosporium* and *Lomentospora*: an updated overview of underrated opportunists. *Medical Mycology*, 56, S102–S125
<https://pubmed.ncbi.nlm.nih.gov/29538735/>
- Smedes S.L., Miller P.E., Dubielzig R.R. 1992. *Pseudallescheria boydii* keratomycosis in a dog. *Journal of the American Veterinary Medical Association*, 200(2):199–202. <https://pubmed.ncbi.nlm.nih.gov/1559876/>
- Thompson K.G., di Menna M.E., Carter M.E., Carman M.G. 1978. Mycotic mastitis in two cows. *New Zealand Veterinary Journal*, 26(7):176–177. <https://pubmed.ncbi.nlm.nih.gov/358027/>
- Tsoi M.F., Kline M.A., Conkling A., Mani R., Corner S.M. 2021. *Scedosporium apiospermum* infection presenting as a mural urinary bladder mass and focal peritonitis in a Border Collie. *Medical Mycology Case Reports*, 33: 9–13. <https://doi.org/10.1016/j.mmcr.2021.05.003>
- Wen H., Lu C., Yuan Z., Wang X., Su S. 2018. Analysis of gut fungal community of cows with clinical mastitis. *Advances in Microbiology*, 8, 366–377. <https://doi.org/10.4236/aim.2018.85024>

IDENTIFIKACIJA PLESNI IZ GENUSA *SCEDOSPORIUM* IZOLOVANE IZ MLEKA KRAVE SA MASTITISOM

Dubravka MILANOV, Oliver STEVANOVIĆ, Milan ĐILAS, Nevenka ALEKSIĆ

Kratak sadržaj

U ovom prikazu slučaja iznosimo osnovne parametre na osnovu kojih je identifikovan izolat iz mleka krave, plesan roda *Scedosporium*. Prikazane su makroskopske i mikroskopske karakteristike izolata, kao i rezultat identifikacije primenom MALDI-TOF masene spektrometrije. Podaci mogu biti korisni u praktičnom radu laboratorija veterinarske medicine, jer predstavljaju prvi izveštaj o izolaciji i identifikaciji ove vrste u veterinarskoj medicini našeg regiona.

Ključne reči: krava, mastitis, plesni, *Scedosporium*