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Occurrence of *Serratia marcescens* on macrofungi in Hungary

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Abstract

Background and purpose: *The main objective of this paper is to report the occurrence of* Serratia marcescens *(Bizio, 1823), a bacterium on macrofungi and review the data of other collectors in Hungary.*

Materials and methods: *Our mycological research was conducted in ten localities of the North Hungarian Mountain Ranges and in one sampling site of Mezőföld between 2019-2022.*

Results: *Infection of* S. marcescens *was recorded on fourteen fungal species in northern Hungary. In addition, we reported 18 occurrence data of this bacterium by other collectors of Hungary. The infection of* S. marcescens *was highest in autumn. The most abundant infected fungal species was the edible and marketable* Lepista personata *(Fr.) Cooke, 1871. We identified the most fungal species as substrate in the family* Agaricaceae.

Conclusions: *We conclude that the infection of* S. marcescens *of macrofungi was detected independently of life mode and habitat occupied of fungi. However, the seasons influenced the occupancy of this species to varying degrees.*

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INTRODUCTION

The genus *Serratia* belongs to the *Bacteria* domain, strain *Pseudomonadota*, class *Gammaproteobacteria*, order *Enterobacterales*, family *Yersiniaceae (1)*. The genus *Serratia* represents a broad and diverse genus of bacterium found in many habitats, such as soil, water, plants, animals, and many *Serratia* species are known as human pathogens *(2, 3)*. Four species of this genus, *S. marcescens*, *S. nematodiphila*, *S. plymuthica* and *S. rubidaea* produce the red pigment prodigiosin *(4, 5, 6, 7, 8)*. Antifungal agent such as oocidin was diagnosed from this genus which was tested mainly for plant pathogens *(9,* 10*, 11, 12, 13, 3)*.

S. marcescens Bizio, 1823 is in the focus of this research. It is a gram-negative bacillus, has been known for two centuries. This species can be easily determined due to prodigiosin insoluble in water. *S. marcescens* was found in 1819 and described in 1823 *(14)*. It is a saprophyte, opportunistic pathogenic, optional anaerobic, biofilm forming bacterium which has been found in many places *(15, 16, 17, 18)*. This species is a member of the normal human intestinal flora recognised as a cause of nosocomial infection *(19, 20, 21, 22)*, such as urinary tract infection, wound infection, arthritis, conjunctivitis, pneumonia and endocarditis *(23, 24, 25)*. The species was researched in patological and food health aspect by several Hungarian publications *(19, 26, 27, 28, 8)*, according to wich,

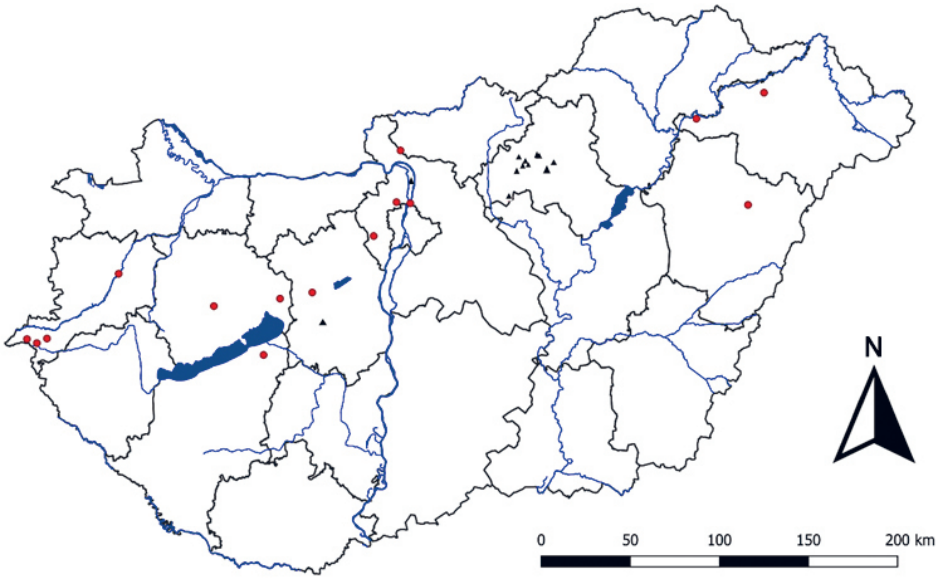

Figure 1. *Sampling sites of fungi parasitised by S. marcescens (▲: own collection data, ●: data of other collectors).*

in some cases the infection can be so large that it causes an epidemic *(29)*. The economic impact of *S. marcescens* is that it can appear in the milk of cattle, as a result of inflammatory symptoms in case of cattles, also consequently on dairy products *(30, 18, 8)*. That's why the bacterium may get in the human body with milk and milk products causing infection. Besides its human and economic aspect, *S. marcescens* is important from mycological and ecological point of view because lot of publications report this bacterium on fungi. According to research, *S. marcescens* can destroy the hypha *(31, 32, 33)*, its activity does not decrease in cold weather *(34)*. It was supported by our observations, too.

Fungi provides a source of food and living space for many species. Besides vertebrates *(35)* and invertebrates such as flies, mosquitoes, macrothermic species *(36)*, fungi may be parasites of other fungi *(37, 38)*. In case of *Coprinus comatus* the parasitic species *Psathyrella epimyces* is a macrofungal species, too. In most cases the parasitic species are microfungi, the most frequent of them the members of genus *Hypomyces (37)*. Many publications report about bacteria on fungi, besides *S. marcescens Burkholderia, Stenotrophomonas, Achromobacter, Lysobacter*, *Pseudomonas*, *Agrobacterium, Cohnella* and *Variovorax* species was detected on fungi such as *Lyophyllum (39, 40, 41)*. Besides this, *Collimonas fungivorans* antifungal bacterium was detected on *Fusarium* species *(42, 43)*, also *Burkholderia rhizoxinica* bacterium recognized as an endosimbiota of *Rhizopus microsporus* fungal species *(44)*. *Pseudomonas, Xanthomonas, Cytophaga* bacterium use hypha as substrate under ground *(45)*.

Data about the occurrence of *S. marescens* on fungi and their ecological aspect have not been published in Hungary. So, this work can be considered a stopgap.

MATERIALS AND METHODS

Data collection was done in ten localities of the North Hungarian Mountain Ranges and in one sampling site of Mezőföld between 2019-2022 (Figure 1). The most sampling sites are situated in Natura 2000 habitats, designation of which is based on the European Union nature conservation directive, according to this, Natura 2000 areas are devided into Special Protection Area (SPA) and Special Area of Conservation (SAC). The aim of the network is the protection of biodiversity with the restauration of the conservation status of the natural habitats and rare-

Table 1. *Nature conservation value of sampling sites of own collection.*

Habitat	Status of nature conservation
Abasár, Szent Anna Lake	Gyöngyös Sár Hill Nature Reserve, Natura 2000 HUBN20046 SAC
Csány, Csányi fishing lake	–
Domoszló, Tarjánka brook	Mátra Landscape Protection Area, Specially Protected Area, Natura 2000, HUBN10006 SPA
Gyöngyös, Deli Lake	–
Gyöngyös, Nyúlmály	–
Gyöngyössolymos, Cserkő Lake	Natura 2000, HUBN10006 SPA
Soponya, edge of sport field	–
Szentendre, Danube bank	–
Vécs, Tarnóca Brook	–
Vécs, Telki pasture	Planned for protection
Mátrafüred, Pipis Hill	–

and vulnerable species (46). Nature conservation status of sampling sites of our collection was varied, among which, there are nature reserves, specially protected areas and habitats planned for protecion (Table 1).

S. marcescens was determined with LOMO MBC-10, Konus Amber 5032 stereomicroscope and Bresser Researcher Bino light microscope based on identification keys of Bánhegyi *(47)*. Presence of the other 3 *Serratia* species which produced prodigiosin in the samples is not possible because of their habitat preference. *S. rubidaea* was described mainly from sewage, also very few data are from other habitats *(48, 49)*, no data from fungi. *S. plymuthica* mainly lives in water *(48)*, *S. nematodiphila* was detected mostly on *Nematoda (50, 6)*, also its impact on black pepper was examined *(51)*. In contrast, *S. marcescens* has been proved to be able to infect and destroy fungi *(31, 32, 33)*. The identification was made by MALDI-TOF analysis for punctual determination.

Valid latin names of detected fungal species are identified based on Funga Hungarica and Mycobank database *(52, 53)*, and rules of their sales in Hungary are contained in the „107/2011. (XI. 10.) VM regulation of the collection, processing and placing on the market of wild mushrooms for eating". Three samples were treated for conservation in several ways, such as a fungarium was made from an infected sporophore with a modified Herpell-like method (54) laminated for better preservation. Besides this, we made preparations in 70% etyl alcohol, also dry sporophore. The conserved samples and photos of samples were placed in the natural science equipment of the ÉASZC Mátra Forestry Tech, Vocational School. During our study we used the results of other collectors besides, who collected data from several parts of Hungary (Figure 1). These data were assembled from Mushroom Identification and Hungarian Mushroom groups of Facebook. The sampling sites of status of nature conservation are included in Table 2.

Table 2. *Nature conservation value of sampling sites of other collectors.*

Habitat	Status of nature conservation
Berhida, meadow	–
Biatorbágy, Nyakaskő Hill	Nyakaskő Ürgehegy Nature Reserve, Natura 2000, HUDI20017 SAC
Budapest, Danube bank	–
Debrecen, Nagyerdő	Debreceni Nagyerdő Nature Reserve, Natura 2000, HUHN20033 SAC
Kondorfa, Alvég	Őrség National Park, Natura 2000, HUON10001 SAC, HUON10001 SPA
Nagyhalász, oak forest	–
Orfalu	Őrségi National Park, Natura 2000, HUON20018, SAC, HUON10001 SPA
Pilisborosjenő, Fehér Hill	Duna-Ipoly National Park, Natura 2000, HUDI20039, SAC
Pilisborosjenő, black pine forest	Duna-Ipoly National Park, Natura 2000, HUDI20039, SAC
Pilisborosjenő, Köves Hill	Duna-Ipoly National Park, Natura 2000, HUDI20039, SAC
Sárvár, oak forest	–
Siófok, Töreki	Natura 2000, HUDD20064, SAC
Szalafő-Papszer	Őrségi National Park, Natura 2000, HUON20018 SAC, HUON10001 SPA
Székesfehérvár, pinus sylvestris forest	–
Szokolya, oark forest	–
Tiszalúc – Tiszadob	Kesznyéten Landscape Protection Area, Natura 2000, HUBN10005 SPA
Úrkút, edge of road	–

RESULTS

We detected 15 occurrence data of *S. marcescens* from 9 habitats of the Nothern Hungarian Mountain Ranges and 1 habitat of Mezőföld on 9 fungal species between 2019-2022. Based on our data, it appeared on fungal species of 5 families exclusively in the second half of the year, 1 of them in September, 10 in October, 4 in November and 1 in December. The most abundant infected fungal species was *L. personata* (Fr.) Cooke, 1871), which has 5 specimens were found (Figure 2). Five fungal species were found in protected area, two species of which, *Byssomerulius corium* (Pers.) Parmasto, 1967 and *Pleurotus ostreatus* (Jacq.) P. Kumm, 1871 were detected in the especially protected Tarjánka Brook Valley. We identified 6 edible and marketable fungal species, 1 edible and 2 not edible fungal species. At most sampling sites, *S. marcescens* was found on only one fungal species, but in Vécs it was de-

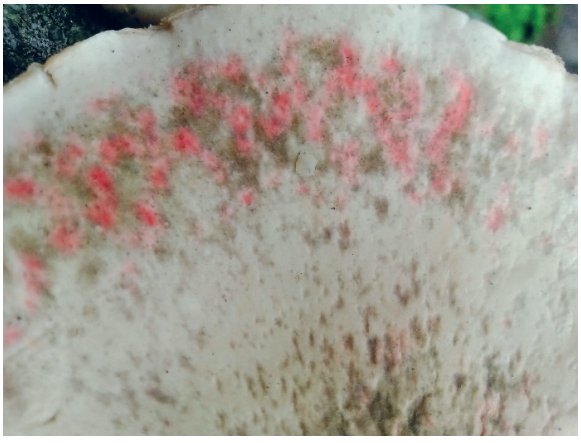

Figure 2. *S. marcescens infection on L. Personata.*

Table 3. *Fungal species of own collection and their dietary value, also date and place of collecting.*

Fungal species	Dietary value	Date of collection	Place of collection
Agaricaceae			
Agaricus arvensis Schaeff., 1774	edible, marketable	02. 10. 2022	Vécs, Tarnóca brook
		17. 10. 2022	Abasár, Szent Anna Lake
A. bitorquis (Quél.) Sacc., 1887	edible, marketable	02. 10. 2022	Vécs, Tarnóca brook
A. crocodilinus Murrill, 1912	edible, marketable	30. 10. 2022	Csány, Csányi fishing lake
Leucoagaricus leucothites (Vittad.) Redhead, 2023	edible	15. 10. 2020	Soponya, edge of sport field
		23. 09. 2022	Gyöngyös, Nyúlmály
Phanerochaetaceae			
Byssomerulius corium (Pers.) Parmasto, 1967	not edible	06. 11. 2022	Domoszló, Tarjánka brook
Pleurotaceae			
Pleurotus ostreatus (Jacq.) P. Kumm, 1871	edible, marketable	06. 11. 2022	Domoszló, Tarjánka brook
Russulaceae			
R.atropurpuurea (Krombh.) Britzelm, 1893	not edible	07. 10. 2022	Gyöngyössolymos, Cserkő lake
R. vesca, Fr. 1836	edible, marketable	25. 10. 2019	Szentendre, Danube bank
Tricholomaceae			
L. personata (Fr.) Cooke, 1871	edible, marketable	28. 12. 2019	Gyöngyös, Deli lake
		29. 10. 2022	Mátrafüred, Pipis hill
		30. 10. 2022	Vécs, Tarnóca brook
		30. 10. 2022	Csány, Csányi fishing lake
		02. 11. 2022	Vécs, Telki pasture

Figure 3. *S. marcescens infection on P. ostreatus.*

Figure 4. *S. marcescens infection on R. atropurpurea.*

tected on 2 *Agaricus* species and *L. personata,* also in Domoszló on 2 species, on *B. corium* and *P. ostreatus* (Table 3) (Figure 3). Regarding the life form of the fungal species, there were present in the samples 2 mycorrhizal-forming, 5 soil-dwelling saprotrophs, 1 tree-living saprotrophs, 1 necrotrophs *(55, 52)*. Substrate fungal species are classified into 2 groups, 1 species, *Russula atropurpuurea* (Krombh.) Britzelm, 1893 in the endangered (VL:3) (Figure 4), and 8 are in the no endangered category *(55, 52)*.

Table 4. *Fungal species of other collectors and their dietary value, also date and place of collecting and name of collectors.*

Fungal species	Dietary value	Date of collection	Place of collection	Name of collector
Agaricaceae				
Macrolepiota excoriata (Schaeff.) Wasser, 1978	edible	18. 11. 2019	Berhida	Vidra Gyula
Amanitaceae				
Amanita citrina Pers., 1797	toxic	19. 10. 2022	Biatorbágy, Nyakaskő hill	Kottyán Péter
		28. 10. 2022	Orfalu	Fiedler Rita
		09. 12. 2014	Pilisborosjenő, Köves hill	Mihályi Ferenc
Boletaceae				
Boletus edulis Bull., 1782	edible, marketable	09. 11. 2017	Sárvár	Benkő Gábor
		24. 10. 2022	Kondorfa, Alvég	Fiedler Rita
Cortinaceae				
Inocybe geophylla (Bull.) P. Kumm, 1871	toxic	04. 11. 2019	Szokolya	Császár Ernő
Hygrophoraceae				
Cuphophyllus virgineus (Wulfen) Kovalenko, 1989	edible	28. 10. 2022	Szalafő-Papszer	Fiedler Rita
		31. 10. 2022	Úrkút	Nagy Péter
Mycenaceae				
Mycena galericulata (Scop.) Gray, 1821	not edible	24. 10. 2020	Debrecen, Nagyerdő	Tóth Zsófia
Pleurotaceae				
Pleurotus eryngii (DC.) Quél., 1872	edible, marketable	28. 10. 2008	Tiszalúc - Tiszadob	Szűcs Béla
P. ostreatus (Jacq.) P. Kumm, 1871	edible, marketable	23. 11. 2016	Nagyhalász	Balogh Sándor
		13. 12. 2020	Budapest, Duna bank	Szűcs Borbála
		21. 11. 2022	Siófok, Töreki	Farkas Péter
Russulaceae				
Lactarius deliciosus (L.) Gray, 1821	edible, marketable	30. 10. 2017	Székesfehérvár	Talaber Gergely
L. sanguifluus (Paulet) Fr., 1838	edible, marketable	12. 11. 2014	Pilisborosjenő, black pine	Fedor Ilona
Tricholomaceae				
Tricholoma terreum (Schaeff.) P. Kumm, 1871	edible, marketable	12. 11. 2014	Pilisborosjenő, black pine	Fedor Ilona
		12. 11. 2014	Pilisborosjenő, Fehér hill	Mihályi Ferenc

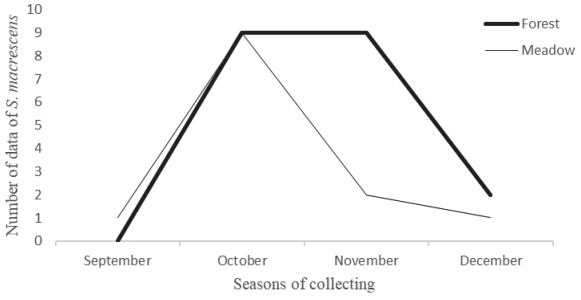

Figure 5. *Distribution of collecting data of S. macrescens on macrofungi in forest and meadow based on collection seasons.*

In addition, 18 occurrence data of this bacterium collected by other collectors were reviewed. They found this bacterium on 11 fungal species from 17 habitats of Hungary (Table 4).

Summarizing data, 33 occurrence data of *S. macrescens* were detected from 19 substrates in Hungary. In total, 9 mycorrhizal-forming, 6 soil-dwelling saprotrophs, 2 tree-living saprotrophs, 2 necrotrophs are known. Considering all data of *S. marcescens*, 3 endangered fungal species (VL:3) and 3 vulnerable fungal species (VL:4) were detected with infection of bacterium. Regarding habitats, 13 samples are from meadows and 20 from forests, the difference between two habitat types was remarkable in November (Figure 5). Data were collected from 13 Natura 2000 areas, 2 landscape protection areas, 3 nature reserves, 1 specially protected area, 6 national parks and 1 habitat planned for protection.

CONCLUSIONS

Significant occurrence of *S. marcescens* bacterium was detected from macrofungi in northern Hungary and was reviewed occupancy in Hungary. The collecting was started in 2019, but the earliest data of Hungary are from year 2008. Sampling was conducted in 3 counties, so based on all observations, occurence data are detected from 9 counties in Hungary. With our 18 data presently 33 occurrence data of *S. macrescens* are known, and in total, 10 families of 19 fungal species as substrate have been observed. The most abundant species was the *L. personata,* appeared exclusively in our own sampling as substrate species (Table 3). The reason for this may be that collectors are not specifically searching for *S. marcescens*; therefore, the substrate was not collected. Our observations support that, in the case of sporophore of fairy-ring most of the parasites concentrate in few hosts *(56)*. It was noticeable in case of *L. personata* in Telki pasture, where *S. marcescens* appeared on 3 sporophore which were close to each other. According to all data, the life mode of the infected fungal species was very different. Based on this it can be said, *S. marcescens* chooses the fungal species independently of its life mode. All occured data show that, the threat of fungal species in Hungary is also different. Besides this, our observations support that the forest fungi were dominant, but the difference was not significant. The observation of *S. marcescens* on fungi in conservation aspect is not negligible because based on our data, neither the habitat type nor the substrate species affects its occurrence. Also, it can appear on endangered and rare fungal species and nature conservation areas.

Because fungi are collected mainly for food purposes, the observation and examination of this bacterium which may cause diseases and in some cases epidemic can be significant. Due to the lack of literature, transmission from fungus to humans is not proven, but evidently not impossible. Others *(30, 8)* report on appearance of this bacterium in cattle milk and the resulting dairy products. We conclude from this, *S. marcescens* as a member of the intestinal microbiome *(19)* can enter the human body through contact and eating in quantities causing diseases. Based on human health literatures *(23, 24, 25)*, it can cause serious diseases in people with weakened immune systems.

In conclusion, it can be said, besides human health activity *S. marcescens* is able to settle in many habitats of Hungary and several fungus species independently of their life mode. Based on this, the fungal identification test has great importance in order to select the infected sporophore and to prevent possible diseases resulting from it. *S. marcescens* appeared in several protected areas of Hungary, also on endangered species. However, based on our observation, their appearance on endangered and rare fungal species is not significant. More research is necessary in order to detect the possible infection of endangered fungal species.

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