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FUNGAL MICROBIOTA OF EDIBLE ROSE FLOWERS

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Abstract

Flowers provide a diverse and unique habitat for microorganisms. A preliminary study on the microbiota of edible rose flowers was carried out at the USAMV of Bucharest. Rose flowers from cultivars Brother Cadfael, Crown Princess Margarett, and Falstaff were collected in October 2023 and May 2024. The composition of the fungal community associated with organic edible rose petals and stamens was analyzed. The fungal community associated with rose petals was represented by isolates of Alternaria spp. (A. alternata), Aspergillus spp. (A. niger, Aspergillus Nigri Section), Aureobasidium spp. (A. pullulans), Cladosporium spp. (C. cladosporioides), Botrytis cinerea, Epicoccum spp. (E. purpurascens), Fusarium spp. and Penicillium spp. This study provides important insights into the fungal community associated with organic edible rose flowers.

Key words*: Brother Cadfael, Crown Princess Margarett, Falstaff, fungal microbiota.*

INTRODUCTION

Plants live in close association with beneficial and pathogenic microorganisms, which together form the plant microbiota. The complex relationship between plants and their microbiota has raised fundamental questions about plant responses to these microorganisms and the main factors determining the microbiota's structure, diversity, and functionality in the soil, rhizosphere, and plant organs (Andrews & Harris, 2000).

Interest in the flower microbiome has recently intensified, but flowers colonizing microorganisms have been studied for over a century. Bacteria and fungi associated with the floral microbiome have very interesting ecological traits that influence their ability to colonize that niche, to make possible associations with flower visitors, including pollinators, and to metabolize unique nutrient sources in flowers (Vanette, 2020)

Due to their ephemerality and complex structure, flowers provide unique habitats for microorganisms, encompassing a variety of distinct niches at the microscale (Aleklett et al., 2014; Keller et al., 2021).

Before anthesis, flower buds and nectar in unopened flowers can contain detectable colonies of fungi and bacteria (Shade et al., 2013; von Arx et al., 2019). Petals of newly opened flowers also contain detectable and culturable microbial communities (Junker et al., 2011).

Flower stigmas and hypanthia of newly opened blossoms also yield culturable bacteria and fungi, with a low incidence (Pusey et al., 2009). Different groups of microorganisms are found on or in flowers. Among the environmental ones, those observed on all flower tissues were *Micrococcus*, *Aspergillus*, and Penicillium, with low abundance and an unknown effect on flower phenotype (Morris et al., 2020; Shade et al., 2013).

Among the microorganisms found on plant surfaces, *Pseudomonas*, *Aureobasidium*, *Rhodotorula*, and *Cryptococcus* have been reported (Farré-Armengol et al., 2016).

Plant pathogens and plant endophyte are represented by isolates of bacteria (*Erwinia*, *Xanthomonas, Streptomyces*) and fungi as *Alternaria*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Monilinia*, and *Microbotryum* (An et al., 2020; Kim et al., 2019).

Flowers are likely the primary habitat for *Acinetobacter*, *Rosenbergiella*, *Metschnikowia reukaufii*, and *Metschnikowia gruessi*. These bacteria and fungi are predominantly isolated from flowers or flower-related habitats. They are numerically dominant in nectar, pollen, and pistils and contribute to the abundant growth and metabolism of nectar and floral resources

(Herrera et al., 2008; Schaeffer & Irwin, 2014; Yang et al., 2019).

The complex, multiple relationships of the plant with pollinators and other flower visitors are also to be considered. Different pollinators can promote or suppress the growth of certain microorganisms colonizing the flowers.

Animals associated microorganisms (commensal or beneficial) that are found in both flowers and animals and which use floral resources as petals, nectar, and pollen are bacteria (*Lactobacillus*) and fungi (*Kodamaea, Metschnikowia, Starmerella, Wickerhamiella*). These communities are not often isolated or frequently numerically dominant in flowers (McFrederick et al., 2012). Some can metabolize floral resources, while others may have a short life span on flowers.

Current understanding of which, when, and how microbial communities form on and within flowers, how associated microbiomes affect floral phenotypes and plant-pollinator interactions, and how these communities disperse out of flowers is reviewed by Vanette (2020). Floral traits, such as morphology and age (Morris et al., 2020), plant nutrient availability, plant chemistry, volatile and non-volatile (Boachon et al., 2019) as well as the presence of visitors, arrival order of flowers visitors (Morris et al., 2020; Vannette & Fukami, 2017) or environmental exposure (Figueroa et al., 2019) and environmental drivers as pollutants, chemicals (Schaeffer et al., 2017) can influence microbial growth on flowers.

In this context, our studies focus on exploring the cultivable microbiota of edible rose flowers, with the aim of better understanding the composition of these microbial communities.

MATERIALS AND METHODS

In October 2023 and May 2024, flowers were harvested from the three varieties of edible roses from the USAMV Bucharest orchard: Brother Cadfael, Crown Princess Margarett, and Falstaff (Figure 1).

Brother Cadfael rose variety is characterized as a vigorous bush with heights of 2.5-3m, large flowers with pink petals (Figure 2), and remontant flowering that can last until the beginning of November.

Crown Princess Margarett is a climbing rose with apricot-coloured petals (Figure 2) and a strong fruity scent. It has vigorous growth, reaching 3.5-4 m in height, single flowers or inflorescences, and remontant flowering.

Falstaff is a climbing rose, with carmine-red petals (Figure 2) and a strong scent of old roses. It has a vigorous bush, reaching 250-300 cm in height, remontant flowering, and large flowers with many petals.

Figure 1. Edible rose plantation at USAMV Bucharest

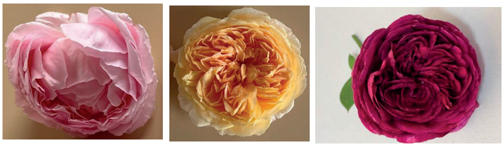

Figure 2. Edible rose varieties: Brother Cadfael, Crown Princess Margaret, and Falstaff

The detection, isolation, and identification of rose flower microbiota were done by incubating petals and stamens in a PDA culture medium (Potato Dextrose Agar, Scharlau).

Petal samples sectioned into small fragments (0.5/0.5 cm) and stamens were placed in Petri dishes on PDA culture medium (Scharlau) and incubated at 22°C.

Colonies developed were identified based on their morphology, followed by microscopic examination. Results are expressed as the incidence (%) of identified isolates (the number of isolates compared to the total number of analyzed fragments) and the incidence of bacterial or fungal colonies (%).

RESULTS AND DISCUSSIONS

In October 2023, bacteria and fungi represented the cultivable microbiota of the Brother Cadfael variety's petals. Compared to the total number of colonies, the incidence of bacteria was 65.6%. Fungi recorded an incidence of 34.4%. The incidence of detected isolates is shown in Figure 3. Thus, the high incidence of bacterial colonies (80%) is highlighted.

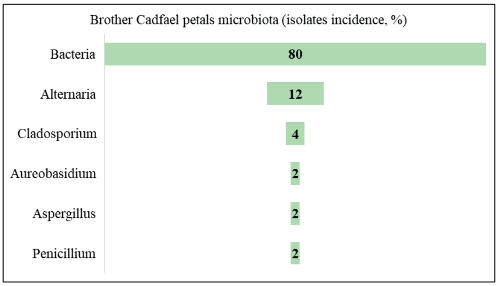

Figure 3. Petal microbiota (incidence of isolates, %), Brother Cadfael variety (October 2023)

Among the detected and identified fungal colonies, those belonging to the genus *Cladosporium* were the majority (24%), followed by those of *Alternaria* (12%). Isolates of the genera *Aspergillus* and *Penicillium* were also identified, with an incidence of 2%, and isolates of the genus *Aureobasidium*.

In samples taken in May 2024, no colonies developed from the incubated fragments were detected.

The microbiota of the colonies detected in Brother Cadfael petals is shown in Figure 4.

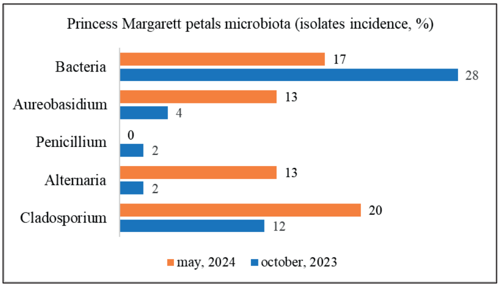

Figure 4. Brother Cadfael petals microbiota in October 2023

In October 2023, the microbiota associated with flower petals of the Crown Princess Margarett variety was represented by isolates from the genera *Cladosporium* (12% incidence), *Aureobasidium* (4% incidence), *Alternaria*, and *Penicillium* (2% incidence). Bacterial colonies were also detected with a 28% incidence (Figure 5). In May 2024, isolates from the genera *Cladosporium* (20% incidence), *Aureobasidium* (13% incidence), and *Alternaria* (13% incidence) were detected.

Some aspects of the detected isolates' morphology are shown in Figure 6.

Figure 5. Petals microbiota, Crown Princess Margaret variety (October 2023 and May 2024)

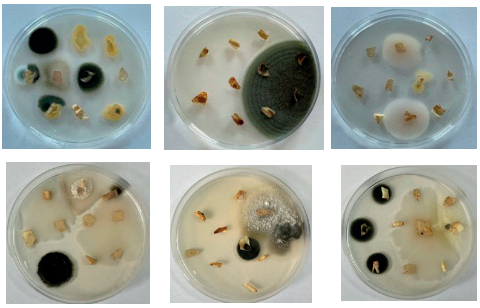

Figure 6. Crown Princess Margaret petals microbiota

In October 2023, the petals microbial community of the Falstaff variety was generally represented by bacterial colonies, with an incidence of 70% (Figure 7). In May 2024, the microbiota was represented by isolates from the genera *Cladosporium* (33% incidence), *Alternaria* (30% incidence), and *Aureobasidium* (3% incidence). Bacterial colonies were also detected (17% incidence). The morphology of some of the colonies detected in the Falstaff petals microbial community is shown in Figure 8.

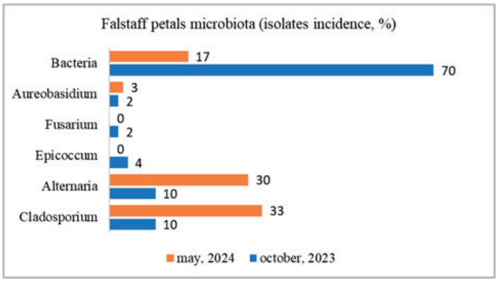

Figure 7. Petal microbiota, Falstaff variety

Aureobasidium, *Fusarium*, *Epicoccum*, *Alternaria*, and *Cladosporium* isolates were recorded among the fungi.

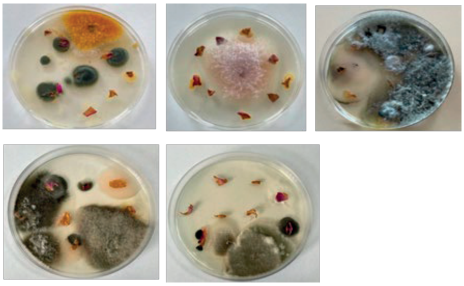

Figure 8. Falstaff petals microbiota in October 2023

The results regarding the microbiota detected and identified at the stamen level are presented in Table 1. In the samples analyzed in October 2023, no significant differences in microbial community were observed between the three tested varieties. Generally, the microbiota was represented by bacterial and fungal colonies, like in the petal variants. The presence of *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, and *Botrytis* colonies was recorded among the colonizing fungi. We report the presence of isolates of *Botrytis cinerea* at the stamen levels, the pathogen known to attack the flowers.

The microflora was less represented in the May 2024 samples. Thus, no colonies developed from the incubated fragments in the Brother Cadfael variety.

Only colonies of the *Cladosporium* genus were detected in the Crown Princess Margaret variety. In the Falstaff variety, colonies of the *Alternaria*, *Aureobasidium*, and *Cladosporium* genera and bacterial colonies have been developed.

In October 2023, the petals microbial community of the Falstaff variety was mainly represented by bacterial colonies, with an incidence of 70% (Figure 7).

Table 1. Rose stamen microbiota

Microbial community	Brother Cadfael		Crown Princess Margaret		Falstaff	
	Oct. 2023	May 2024	Oct. 2023	May 2024	Oct. 2023	May 2024
Fungi						
Alternaria	🟨		🟨		🟨	🟩
Aspergillus					🟨	
Aureobasidium			🟨			🟩
Cladosporium	🟨		🟨	🟩	🟨	🟩
Botrytis	🟨				🟨	
Penicillium	🟨		🟨			
Bacteria						
Bacteria	🟨		🟨		🟨	🟩

The morphology of the colonies detected in October 2023 is shown in Figure 9.

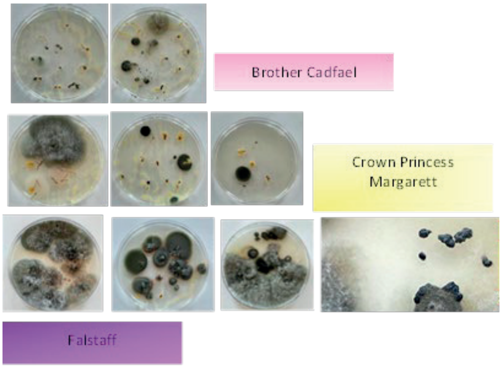

Figure 9. Rose stamen microbiota

Our research highlighted the cultivable microbiota of organic edible rose flowers at the level of petals and stamens.

The fungal microbial community associated with the petals was represented by isolates belonging to the genera *Alternaria* (*A. alternata*), *Aspergillus* (*A. niger*), *Aureobasidium* (*A. pullulans*), *Cladosporium* (*C. cladosporioides*), *Botrytis* (*B. cinerea*), *Epicoccum* (*E. purpurascens*), *Fusarium* and *Penicillium*. Bacterial colonies were the majority in October in all the tested varieties (Brother Cadfael, Crown Princess Margaret, and Falstaff).

Isolates of *Alternaria* and *Cladosporium* have been detected to have a higher incidence on petals. Also, they were detected in stamens in autumn (October 2023). *Alternaria*, *Cladosporium*, and *Botrytis* species can colonize plant tissues asymptomatically or symptomatically. They have been reported on petals, nectar, pollen, and pistils, and their presence can increase flower attractiveness. Their abundance is variable, typically low (An et al., 2020; Kim et al., 2019).

Species of *Cladosporium* (*Cladosporium cladosporioides*, *C. devikae*, *C. macadamiae*, and *C. proteacearum*) are reported as associated with flower blights (Van den Berg et al., 2008; Prasannath et al., 2021).

Isolates of *Aspergillus* (*A. niger*, *Aspergillus* Nigri species complex) and *Penicillium* have been recorded with low incidence on petals. Their presence on stamens has also been highlighted (Morris et al., 2020; Shade et al., 2013).

Among fungal isolates, those belonging to the genera *Aureobasidium and Epicoccum* may be interested in understanding their role in the flower microbiota. These genera are known for their species, which have potential in the biological control of plant diseases.

Isolates of *Aureobasidium* are known to be present at the petals levels, impacting metabolizing plant compounds and producing volatiles or other metabolic by-products (Farré-Armengol et al., 2016). Species of the genus *Aureobasidium* produce different compounds (pullulan, β-glucan, malic and polymalic acids, melanin, lipids) used in agriculture. They are also known for their effect on plant growth and their protection. Isolates of *Aureobasidium* adhere to plant cells, producing extracellular polysaccharides, forming biofilm (Rensink et al., 2024). On the market, Blossom Protect and Botector are bioproducts formulated based on *A. pullulans* strains DSM 14940 and DSM 1494 (Andermatt Biocontrol Romania) highly effective for the prevention of fire blight in pome fruits through colonization of blossoms and nectarines and natural competition for space and nutrients or grey mould infections on grapes and berries.

Epicoccum species are ascomycetes known for their endophytic potential, which promotes plant growth and protects plants from other pathogens (Favaro et al., 2012; de Cal et al., 2009).

It can be assumed that the presence of *Aureobasidium* and *Epicoccum* isolates in the flower microbial community may be linked to controlling natural pathogens. Further studies are needed to examine whether this community diversity, abundance, and/or species composition could enhance protection against floral pathogens and which biotic or abiotic conditions affect microbiome-mediated pathogen protection. Research is ongoing to characterize *Aureobasidium* and *Epicoccum* isolates in terms of antagonistic potential.

CONCLUSIONS

The research highlighted the cultivable microbiota associated with the flowers of organic edible roses (petals and stamens) in autumn (October) and spring (May). To our knowledge, this is the first report of a flower's microbial community.

Alternaria, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Botrytis*, *Epicoccum*, *Fusarium*, and *Penicillium* isolates represented the fungal microbial community associated with edible rose petals. A high incidence of bacterial colonies was recorded in all tested varieties (Brother Cadfael, Crown Princess Margarett, and Falstaff).

Alternaria, *Aspergillus*, *Aureobasidium*, *Cladosporium*, and *Botrytis* isolates represented the stamen microbiota. Our research highlights the presence of isolates of *Botrytis cinerea* at the stamen level.

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