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EXPERIMENTAL ARTICLES

Seasonal Dynamics in a Yeast Population on Leaves of the Common Wood Sorrel *Oxalis acetosella* L.

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Abstract—Analysis of an epiphytic yeast population on the leaves of the evergreen common wood sorrel *Oxalis acetosella* L. throughout a year showed that the density and the species composition of this population underwent regular seasonal changes. There were almost no yeasts on the young spring leaves. However, the yeast population on the mature leaves tended to increase in the autumn, reaching a maximum after the formation of continuous snow cover. Then the yeast population on the leaves tended to decrease, reaching a minimum in the spring. The species diversity of the yeasts was maximum in the autumn. The population of the epiphytic yeast species *Cystofilobasidium capitatum, Rhodotorula fujisanensis, Leucosporium scottii*, and *Cryptococcus flavus* peaked in the autumn. On the other hand, the population of the widespread epiphytic species *Cryptococcus laurentii* on the wood sorrel leaves peaked in January. The relative abundance of the red-pigmented phytobionts *Rhodotorula glutinis* and *Sporobolomyces roseus* virtually did not change throughout the year. The relative abundance of the euribiotic species *Cryptococcus albidus* showed irregular monthly variations. The data obtained show that the epiphytic microbial population of various plants can be comprehensively studied only by analyzing this population throughout the vegetative period of the plants.

Key words: yeasts, phyllosphere, *Oxalis acetosella*, epiphytic microorganisms, seasonal dynamics.

Plant leaves are populated by many microorganisms, including saprotrophs, phytopathogens, commensals, mutualistic symbionts, endophytes, and accidental species [1]. The epiphytic complex is represented by eccrisotrophic bacteria, mycelial fungi, and yeasts, which consume plant exudates [2].

The phyllosphere is a habitat of many yeasts. Epiphytic yeasts were first described by di Menna [3]. The abundance and the taxonomic composition of epiphytic yeast communities have been studied in various climatic zones [4–11]. The most frequent phyllosphere species are the basidiomycetous yeasts *Cryptococcus laurentii, Cr. flavus, Rhodotorula fujisanensis, R. glutinis, R. mucilaginosa, R. graminis*, and *Sporobolomyces roseus.* Epiphytic yeasts possess specific adaptive properties, such as carotenoid pigmentation, the presence of polysaccharide capsules, the ability to form chlamidospores and chlamidospore-like cells, and the production of ballistospores. Such yeasts are assumed to be a specific life form of phytobionts [12].

The development of epiphytic yeasts on plant leaves depends on many factors, including the composition of plant exudates, temperature, humidity, solar radiation, and the physiological activity of plants. Most of these factors undergo heavy seasonal and ontogenetic variations, which may cause temporal dynamics in the species composition of epiphytes [13]. The study of this dynamics can provide insight into the role of microbial associations in the life of higher plants, particularly the evergreen plants of temperate zones, whose leaves are exposed to contrasting levels of environmental factors in the process of plant ontogeny.

Although this problem has been the subject of a good deal of investigations, the data obtained are contradictory [10, 11, 14–16] and can hardly be generalized. For instance, the population density and the diversity of yeasts on the leaves of deciduous plants were found to be higher in autumn than in spring [11, 15], whereas the yeast population on the leaves of evergreen plants unexpectedly exhibited almost no seasonal variation [11].

The aim of this work was to study annual dynamics in the population and diversity of epiphytic yeasts on the leaves of one plant species growing in the temperate zone with contrasting seasonal levels of environmental factors.

MATERIALS AND METHODS

Experiments were carried out with the common wood sorrel *Oxalis acetosella* L. This plant species was chosen for its ubiquitous presence in the coniferous and mixed forests of the temperate zone and for its ability to retain green leaves of different ages through the winter into the following summer, which allowed us to perform the analysis of the epiphytic yeasts of this plant all

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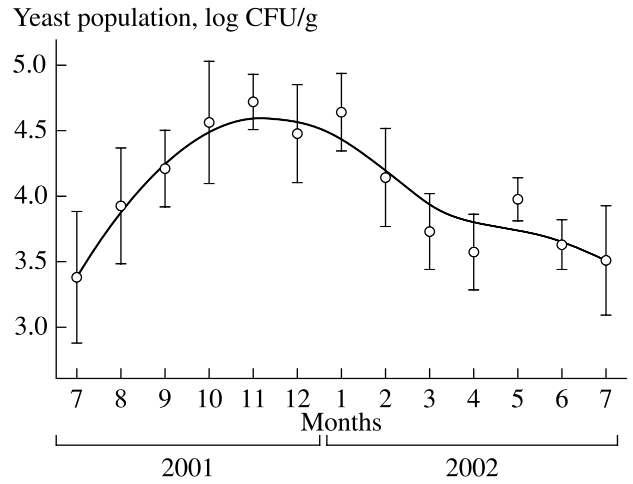

Fig. 1. The annual dynamics of epiphytic yeasts on leaves of the common wood sorrel *Oxalis acetosella* L. Shown are the average monthly values (open circles), the confidence intervals estimated for $P = 0.95$ (error bars), and the best-fit smoothed curve through the data points.

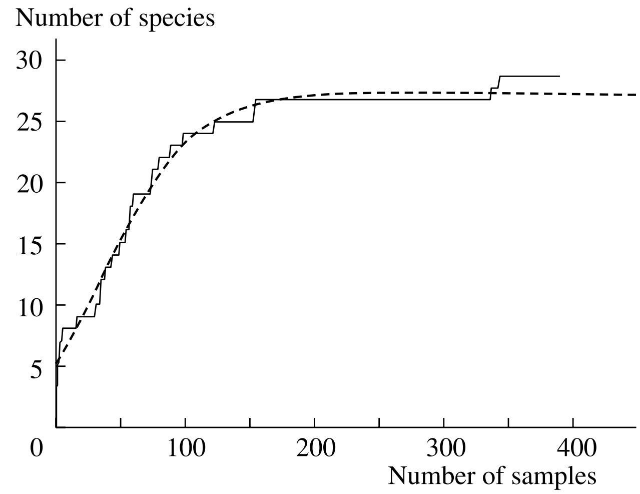

Fig. 2. The number of yeast species identified in the *Oxalis acetosella* L. phyllosphere as a function of the number of leaf samples analyzed.

year round. The mature wintered leaves were studied along with the new leaves that emerged in the spring.

Green wood sorrel leaves were collected in a birch forest dominated by the growth of hairlike sedge, which was located on the territory of the Losiny Ostrov Reserve, Moscow. The leaves were sampled 55 times (2–3 times a week) from July 2, 2001, through July 3, 2002. From November 15, 2001, through March 7, 2002, the leaves were collected from under the covering snow. Each of the 55 groups of leaf samples included 8–10 mixed samples, each containing about ten wood sorrel leaves. The leaf samples were analyzed for inhabiting yeasts on the day of sampling.

To enumerate and classify the inhabiting yeasts, leaf samples (0.1–0.6 g) were flooded with 50 volumes of distilled water and disintegrated for 3 min by using a tissue homogenizer. The leaf homogenate was plated in triplicate onto malt extract agar, which was acidified to pH 4–4.5 with lactic acid to suppress bacterial growth. The agar plates were incubated at 5–10°C for 7 to 14 days. The grown yeast colonies were examined for different colonial morphotypes by using a binocular magnifying glass. The colonies of each morphotype were enumerated, and two to three of them were isolated in pure culture and identified to a species level according to morphological and physiological identification criteria [17, 18]. The necessary standard media were purchased from Difco.

The total yeast population and the abundance of each yeast species were expressed in colony-forming units (CFU) per gram dry weight leaf.

Such ecological parameters as temperature, insolation, humidity, etc., at the time of sampling were not measured. Changes in the epiphytic yeast population were referred to seasons only.

RESULTS AND DISCUSSION

The average annual yeast population on the wood sorrel leaves was found to be 3.8×10^3 CFU/g dry wt leaf, or approximately 500 CFU/leaf. The yeast population showed statistically significant seasonal variations (Fig. 1). There were almost no yeasts on the new spring leaves. The yeast population reached a detectable level (about 10^2 CFU/g) only on relatively mature leaves. In July 2001, the yeast population on the leaves was 6×10^3 CFU/g and then gradually increased to reach a maximum (7.1×10^5 CFU/g) in November 2001. From January 2002 on, the yeast population tended to decrease and reached a minimum (ca. 10^3 CFU/g) in July 2002.

The yeasts found on the wood sorrel leaves were classified into 27 species (8 ascomycetous and 19 basidiomycetous species) belonging to 12 genera (table). This level of yeast diversity is likely to be the maximum on the wood sorrel leaves, as is evident from the graph showing the number of detected yeast species as a function of the number of leaf samples analyzed (Fig. 2). During the analysis of the first 100 leaf samples, new yeast species were detected at a mean rate of one species per 5 or 6 samples analyzed. However, the analysis of the last 388 leaf samples (from the 150th on) yielded only two new species.

The most frequent species in the wood sorrel phyllosphere were the imperfect basidiomycetous yeasts *Cr. laurentii, Cr. flavus, R. glutinis*, and *S. roseus*, which are widespread in different geographic zones on the green and dead part of plants and belong to typical phytobionts [12].

In addition to the phytobionts, the wood sorrel leaves also accommodated the yeast like fungus *Cysto-*

Yeast species isolated from leaves of the common wood sorrel *Oxalis acetosella* L. and the ecological parameters of the yeasts averaged over a period of observations

Species	Frequency of occurrence, %	Population density, × 10^3 CFU/g	Frequency of domination, %
Rhodotorula glutinis (Fresenius) Harrison	41.0	7.16 ± 1.60	9.54
Cryptococcus albidus (Saito) Skinner	35.3	9.64 ± 1.96	21.91
Sporobolomyces roseus Kluyver et van Niel	33.8	1.12 ± 0.18	6.44
Cryptococcus laurentii (Kufferath) Skinner	24.7	10.94 ± 2.30	18.81
Cystofilobasidium capitatum (Fell *et al.*) Oberwinkler et Bandoni	14.4	1.44 ± 0.46	1.55
Rhodotorula fujisanensis (Soneda) Johnson et Phaff	10.3	1.00 ± 0.30	3.87
Cryptococcus flavus (Saito) Phaff et Fell	9.0	1.45 ± 0.39	4.38
Cryptococcus terricola Pedersen	9.0	1.06 ± 0.33	4.90
Leucosporidium scottii Fell *et al.*	8.5	1.88 ± 0.61	4.38
Cryptococcus magnus (Lodder et Kregervan Rij) Baptist et Kurtzman	5.9	0.08 ± 0.06	2.06
Debaryomyces hansenii (Zopf) Lodder et Kreger-van Rij	4.1	0.39 ± 0.18	2.32
Rhodotorula minuta (Saito) Harrison	3.6	0.08 ± 0.05	1.03
Cryptococcus podzolicus (Bab'eva et Reshetova) Golubev	2.8	0.17 ± 0.08	1.55
Blastobotrys sp.	2.3	0.09 ± 0.05	1.03
Candida sake (Saito et Ota) van Uden et Buckley	1.8	0.06 ± 0.03	1.29
Cryptococcus diffluens (Ruinen) von Arx et Weijman	1.8	0.05 ± 0.04	–
Rhodotorula graminis di Menna	1.8	0.05 ± 0.03	0.77
Rhodotorula mucilaginosa (Jorgensen) Harrison	1.5	0.03 ± 0.02	1.03
Candida sp.	1.0	0.03 ± 0.02	–
Pichia membranaefaciens (Lodder et Kregervan Rij) Wickerham et Burton	1.0	0.05 ± 0.03	0.52
Rhodotorula sp.	1.0	0.38 ± 0.38	–
Trichosporon sp.	1.0	0.05 ± 0.02	–
Debaryomyces vanrijiae (van der Walt et Tscheuschner) Abadie *et al.*	0.8	0.05 ± 0.04	0.52
Metschnikowia pulcherrima Pitt et Miller	0.8	0.18 ± 0.18	0.26
Mrakia frigida (Fell *et al.*) Yamada et Komagata	0.5	0.01 ± 0.01	–
Cystofilobasidium infirmo-miniatum (Fell *et al.*) Hamamoto *et al.*	0.3	0.01 ± 0.01	–
Hanseniaspora occidentalis Smith	0.3	0.04 ± 0.02	–
Rhodotorula hinnulea (Shivas et Rodrigues de Miranda) Rodrigues de Miranda et Weijman	0.3	0.05 ± 0.02	–

Note: The frequency of occurrence was defined as the ratio of the number of leaf samples in which a given species was detected to the total number of leaf samples analyzed, expressed as a percent. The frequency of domination was defined as the ratio of the number of leaf samples in which the population of a given species comprised more than 30% of the total yeast population to the total number of leaf samples analyzed, expressed as a percent.

filobasidium capitatum, which is typical of forest floors and is characterized by relatively high hydrolytic activity and a well-developed mycelium, and the anamorphic basidiomycetous species *Cryptococcus albidus*, which is the most euribiotic organism among the known yeast species. *Cr. albidus* is equally frequent in soils, on plants, and on plant debris in different geographic areas [12]. It should be noted that some yeast strains, preliminarily identified as *Cr. albidus* based on their phenotypic characteristics, were later reclassified on the basis of ribosomal DNA sequence data [19]. Among such yeasts are the species *Cr. diffluens* and *Cr. terricolus*, which were found on the wood sorrel leaves as well (table).

In general, the yeast population on the wood sorrel leaves is not specific and is represented by typical phytobionts, saprobionts, and euribionts, which commonly inhabit the phyllosphere of various plants and plant debris. However, the proportion between these groups of yeasts considerably varied throughout the year (Fig. 3). The species diversity of the yeasts was maximum (four to five species per leaf sample analyzed) in the autumn and in the early winter, whereas it did not exceed one to two species per leaf sample in June and

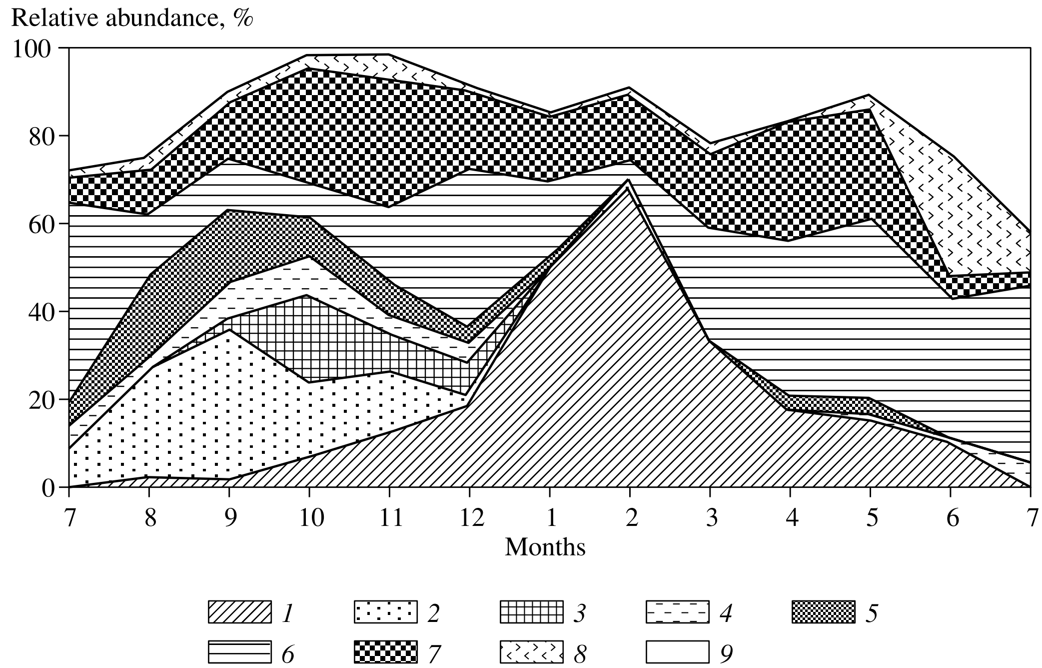

Fig. 3. The annual dynamics of dominant epiphytic yeasts in the common wood sorrel *Oxalis acetosella* L. phyllosphere: (*1*) *Cr. laurentii*, (*2*) *L. scottii*, (*3*) *C. capitatum*, (*4*) *R. fujisanensis*, (*5*) *Cr. flavus*, (*6*) *Cr. albidus*, (*7*) *R. glutinis*, (*8*) *S. roseus*, and (*9*) others.

July. Correspondingly, the Shannon diversity index increased from 0.5 in July to 1.5 in December. From January through the following spring, the yeast diversity again decreased.

The relative abundance of the red-pigmented phytobionts *R. glutinis* and *S. roseus* changed little throughout the year, and that of the euribiotic species *Cr. albidus* showed irregular monthly variations. The other dominant yeast species showed regular seasonal variations. In the autumn, the yeast population on the wood sorrel leaves was diverse and dominated by the species *C. capitatum, R. fujisanensis*, and *Leucosporium scottii.* The population of these three species peaked in September to November and then tended to decrease to be almost zero from January through May. Some strains of these species produced teliospores, which are considered to be the wintering structures of heterobasidial fungi.

In contrast, the abundance of another widespread epiphytic species, *Cr. laurentii,* was at a minimum in the autumn. From November on, its abundance drastically increased to reach a maximum in January. Then it decreased again and reached a minimum in July.

Thus, the population density and the species diversity of the epiphytic yeasts inhabiting the wood sorrel phyllosphere are subject to considerable seasonal variations. The relevant studies of other researchers were performed by collecting leaf samples for analysis no more than one or two times per season [11, 13, 14]. Due to the multiple leaf sampling, we succeeded in revealing regular seasonal variations in the abundance and diversity of the epiphytic yeast population in the wood sorrel phyllosphere.

The data obtained can be generalized as follow: Young leaves contain almost no yeasts. The yeast population on mature leaves tends to increase in autumn, reaching a maximum in winter. Then the yeast population in the phyllosphere tends to decrease, reaching a minimum in spring. The species diversity of yeasts is maximum in autumn, which is manifested both in a greater number of species and in the absence of marked dominants.

The population density of epiphytic yeasts is maximum in November through February, i.e., after the formation of continuous snow cover. This can be accounted for by the fact that young green leaves exude insufficient amounts of nutrients, while mature and aging leaves exude nutrients in sufficient amounts to provide for the growth of a larger yeast population. To the best of our knowledge, there are no data in the literature concerning the effect of the physiological activity of plants on the development of epiphytic yeasts. As for epiphytic bacteria, some data show that their population on mature leaves is greater than on young leaves, whereas other data are indicative of a constancy of the epiphytic bacterial population throughout the vegetative period of plants [16].

The observed increase in the epiphytic yeast population on the wood sorrel leaves in the autumn may also be related to the high moisture content of the surface air and the phyllosphere. This suggestion is in agreement with the data showing that a high air humidity promotes the growth of yeasts on plant leaves [11]. In addition, at the end of the vegetative period, the protective cuticle of leaves may be damaged, thereby making the nutrients of the leaves more available to the epiphytic yeasts.

Of great interest is the fact that seasonal variations in the population parameters of epiphytic yeasts are species-specific. For instance, the population of the epiphytic yeast species *C. capitatum, R. fujisanensis, L. scottii*, and *Cr. flavus* peaked in the autumn, whereas the population of the widespread epiphytic species *Cryptococcus laurentii* inhabiting the wood sorrel leaves peaked in January.

The taxonomic specificity of the phyllosphere epiphytic microorganisms is as yet poorly understood [4, 11]. The data obtained in this study show that the epiphytic microbial population on various plants can be comprehensively investigated only by analyzing this population throughout the vegetative period of the plants. Such studies can also reveal the ecological preferences of typical epiphytic and euribiotic yeast species.

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