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ECOLOGY

Spatial Structure of Epiphytic Yeast Communities on Fruits of *Sorbus aucuparia* L.

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Abstract—The subject of this research is epiphytic yeast communities formed on the surface of *Sorbus aucuparia*. The object is to make quantitative assessment of the yeast communities' differentiation of the same but distant substratum. Results of the nested ANOVA demonstrated that with increase in distances, there are increases in the variation of total number and relative abundance of the dominant yeast communities. The average similarity between groups of single fruits (Sørensen's f Similarity Coefficient) regularly decreased with distance. The results demonstrate that the number and structure of separate yeast groups depend not only on ecological factors but also on proximity to other communities. Such aggregation in the distribution of the microorganisms' species caused by migration and colonial resettlement should be taken into account when analyzing their diversity in natural habitats.

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Soil microbiology confirms an exclusive free-ranging form of microorganisms and the concept of microbial pool (Zvyagintsev, 1987), which even at the beginning of the twentieth century were figuratively expressed through a well-known postulate: *Everything is everywhere, but the environment selects* (Beijerinck, 1912). Therefore, in works on microbial ecology, it is almost always attempted to tie the presence or absence of some species of microorganisms in an analyzed substratum solely to certain factors of the environment: the presence of an accessible feed source or inhibitor growth, temperature, humidity, pH, etc. It is considered that environmental factors certainly define the taxonomic structure of microbial communities. Thereby they differ from communities of vascular plant and animals. Spreading of the species composition is substantially determined by geographical barriers (Finlay, 2002). For example, the structure of fauna and flora of isolated islands depends more on the probability of drift than on the degree of its fitness to the given conditions. In this case, space, the degree of removal of an occupied habitat, is the major cause of species presence.

Questions about role of spatial effects in the course of microbial communities formation are poorly developed in microbial ecology (Chernov, 2001). Nevertheless, it is obvious that the specific structure of ephemeral microbial communities formed on short-lived substrata, rich with readily available feed sources, should, mainly, depend on the probability of initial cell contamination. It is known that insects participate in transferring yeast cells and, thus, invasion ephemeral vegetative substrata (Lachance et al., 2001; Brysch-Herzberg, 2004).

Assessments of the influence of spatial factors on yeast communities taxomical structure determined using modern phylogenetic criteria are very important nowadays. Recently the number of described species of yeast, especially representatives of anamorphous genera *Candida*, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, has increased. Many of them were described mainly on the basis of genotypic criteria. Ecological niches of such formal nominal taxons can be blocked, and they can occupy the same substratum with identical success. First of all this concerns closely related species that do not have a perfect stage, breeding autonomically and extending in a colonial way. The probability of strain detection of such species with identical genotypes should be greater when sampling sites are located close to each other.

The goal of this research was a quantitative estimation of the degree of yeast community differentiation, caused, basically, by the spatial position. For this purpose, we tried to organize an experiment, so that, estimating the influence of spatial affinity, we would maximally exclude the influence of other factors.

MATERIAL AND METHODS

For research were chosen fruits of the mountain ash *Sorbus aucuparia*. This natural substratum met the following requirements: it should be a fully possible standard according biochemical composition, numerous, and various yeast communities should form on it; it should be sufficiently widespread.

Sorbus fruits were sampled in nine geographical points in Russia and Moldova (Table 1). At each point

Table 1. Geographical points of sampling sites

Region	No. of point	Geographical point
Moldova	1	Territory of Institute of Biological Methods of Plant Protection, Kishinev
	2	Petricans microdistrict, territory of Agrouniversity, Kishinev
	3	Botany microdistrict, street of Roses, Kishinev
	4	Territory of Genetic Institute, Kishinev
Russia	5	Moscow State University, Vorob'evy Gory, Moscow
	6	Forest park, Troparevo, Moscow
	7	Surroundings of Dubna, Moscow region
	8	Burzevo country, Moscow region
	9	Central Forest State Nature Biosphere Reserve, Tver region

three *Sorbus* trees were selected, the distance between which was no more than 100 m. Three single fruits were taken from each corymb. The yeast population of each fruit was analyzed separately. Thus, the experiment was initially directed toward processing according to the factorial dispersive analysis, including four hierarchical factors: a fruit, a corymb, a tree, a geographical point. Every factor reflects a certain degree of spatial removal: a few centimeters in the case of fruits in one corymb, a few meters for fruits in different corymbs of a single tree, a few dozen meters for fruits on different trees in one point, and dozens or hundreds of kilometers for different geographical points. Additionally, it is possible to study the factor of *geographical region*, representing climate variation in areas (Russia, Moldova).

To exclude the influence of changes in several yeast groups, samples were collected within deadlines, during September to October. All fruits, with an average weight of 0.5 g, were analyzed within 2–3 days after collecting. Each fruit was placed in a test tube with 1 ml of sterile water, then it was mashed with a sterile glass stick and intensively stirred for 3 min. Every sample was plated twice on malt agar acidified with lactic acid to pH 4–4.5 for bacteria growth suppression. Plate were incubated in a refrigerator for 14–21 days at a temperature of 5–6°C, for delay of filamentous fungi growth. Raised yeast colonies were divided into macromorphological types using a binocular microscope. Every type was considered separately. Two or three cultures representing each type of colony were isolated and purifified, Identification was performed according to morphological and physiological characters using following keys (Barnett et al., 1990; The Yeasts : , 1998). Each sample was characterised by total quantily, expressed in colny forming units (CFU) per gramm of substrate, and by list of isolated species, including their relative abundance. For a quantitative assessment of the yeast variety, Shannon Index of Similarity was used. For similarity assessment between yeast groups, we used Sorensen's Quotient of Similarity for quantitative data:

$$S_{12} = \frac{2\Sigma\min(C1_i, C2_i)}{\Sigma C1_i + \Sigma C2_i},$$

where $C1_i$, $C2_i$ are the relative abundance of the i type in the 1st and 2nd groups.

RESULTS

Yeast was found on practically all analyzed fruits, in all corymbs. The average abundance of yeast was 1.5×10^4 CFU/g, which corresponded to 3×10^4 CFU/g for the average abundance of yeast in the majority of vegetative substrata.

For assessment of the force influence on spatial removal, the aggregate number, variety, and correlation of relative abundance of dominating species, four-factorial dispersive analysis was carried out. Relative abundance and Shannon index were used as dependent variables in the analysis, while categorised variables were fruit, corymb, tree, and geographical point, numbered hieratically. All the factors were treated as random and embedded. So, the gradation of the factor fruit was included in gradation of the factor corymb; there are embedded in gradation of the factor *tree*, etc. The results demonstrated that increased distances enlarged the variation of basic characteristics of yeast community, such as the aggregate number and variety. This is seen from F-test values, which tells us about natural increase with increase of scale (Table 2). Distinctions between the average values of all indicators for different geographical points are authentic. In most cases the influence of the minimum distances between contiguous fruits in one corymb was not statistically significant. These tendencies are accurately seen when traced in the comparison of the average size of each tree through the following points (Fig. 1): the average number of yeast on fruits authentically differs from different points, and distinctions between the average for each tree in one point usually appear questionable.

Similar trends were found from the analysis of the relative abundance of the majority of yeast species. A total of 20 species of yeast and yeast-like fungi were found on fruits of *Sorbus*, among which, as well as on the majority of plant substrats, the following widespread eurybiont and epiphytic species dominated: *Aureobasidium pullulans*, *Cryptococcus* spp. (Filobasidiales, Tremellales), *Rhodotorula glutinis* sensu lato, *Sporobolomyces roseus*. In the minority were *Rhodotorula fujisanensis, R. minuta, R. mucilaginosa, Leucosporidium scottii, Sporidiobolus salmonicolor, Metschnikowia pulcherrima*, and *Guehomyces pullulans*. The species list included taxa corresponding to the modern phylogenetic concept, and so-called phenotypic species which frequently represent a group

Table 2. Variation of degree parameters of the yeast population of *Sorbus* according to gradation of spatial factors: results of F-test according to the results of one-factorial dispersive analyses

Parameter	Fruit	Corymb	Tree	Point
Total quantity, CFU/g	2.2	10.65*	12.31*	74.76*
Number of species to a fruit	1.18	0.39	8.98*	46.04*
Sorensen's Quotient of Similarity	1.17	4.64*	1.21	25.09*
Relative abundance of species				
Cryptococcus spp., (Filobasidiales)	2.12	7.89*	3.62*	28.04*
Aureobasidium pullulans	2.5	1.23	5.68*	75.24*
Sporobolomyces roseus	0.04	2.4	8.89*	5.56*
Cryptococcus spp., (Tremellales)	1.72	0.81	0.55	6.51*
Cystofilobasidium capitatum	0.09	30.75*	2.48	13.58*
Rhodotorula fujisanensis	0.14	2.55	8.35*	25.25*
R. minuta	2.68	1.87	0.27	8.76*
Metschnikowia pulcherrima	2.85	0.1	5.06*	3.05*

Note: Effects are significant when $p < 0.05$.)

closely related to phylogenetic species, which are not distinguishable without application of molecular identification methods. The choice of phenotypic level is connected with the necessity of statistical analysis of a considerable quantity of samples.

The rather high abundance of teleomorphic dimorphic basidiomycete *Cystofilobasidium capitatum*, which usually observed at the late stages of destruction of plant residues or in forest litter, is an interesting feature of the yeast group of *Sorbus* (Bab'eva, Chernov, 1995; Maksimova, Chernov, 2004).

The lists of species, allocated in each studied point, were practically identical. However, correlation of their relative abundance on single fruits, corymbs, and trees strongly varied. It was possible to find 3–4 species (maximum 7) during plating experiment. The same trend as for general indicators was observed for a relative abundance of the dominating taxonomic groups: differences in the average values of abundance between points were higher than between trees in one point, and between the latter, they were higher than between corymbs on one tree. As a rule, differences in the average relative abundance of species on single fruits in one corymb are questionable (Table 2).

The factors of Sorensen's Quotient of Similarity were calculated for assessment of the similarity of yeast groups between all pairs of analyzed fruits. The resulting matrix of similarity was divided into parts according to the following variants: fruits in one corymb, fruits in different corymbs of one tree, fruits of different trees in one point, fruits in different points of one geographical region, fruits in different geographical regions (Russia or Moldova). For each part, the average value of Sorensen's Quotient of Similarity was calcu-

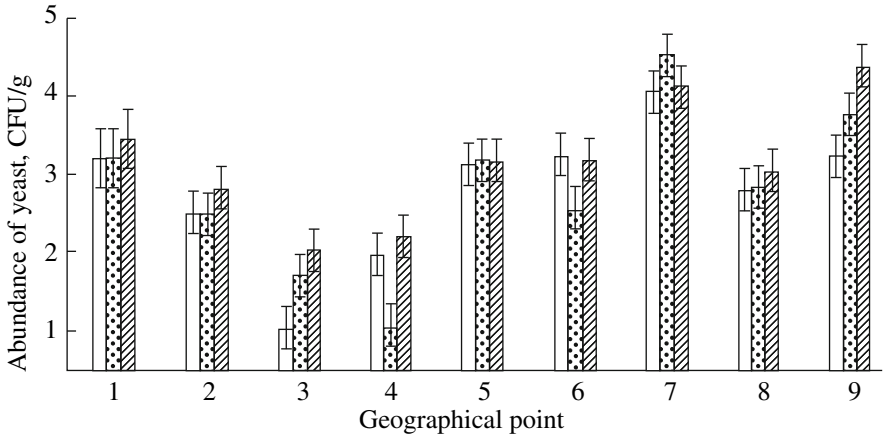

Fig. 1. Average number of yeast on *Sorbus* fruits on three trees in each geographical point (Table 1).

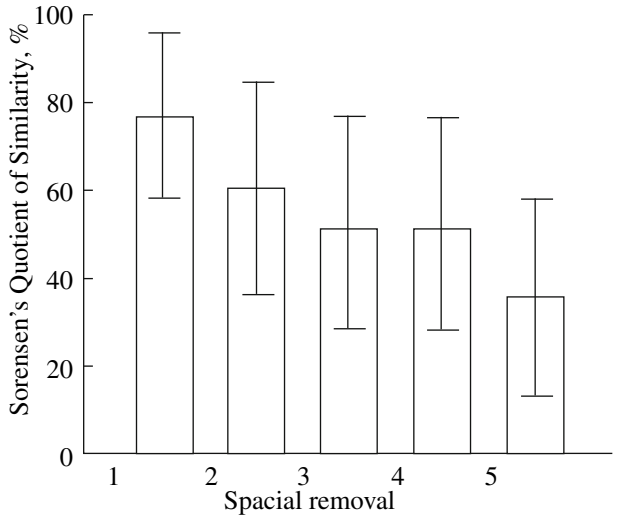

Fig. 2. Average according to Sorensen's Quotient of Similarity among yeast groups on *Sorbus* fruits: (1) in one corymb, (2) in different corymbs of one tree, (3) on different trees in one geographical point, (4) in different geographical points in one region, (5) in different regions.

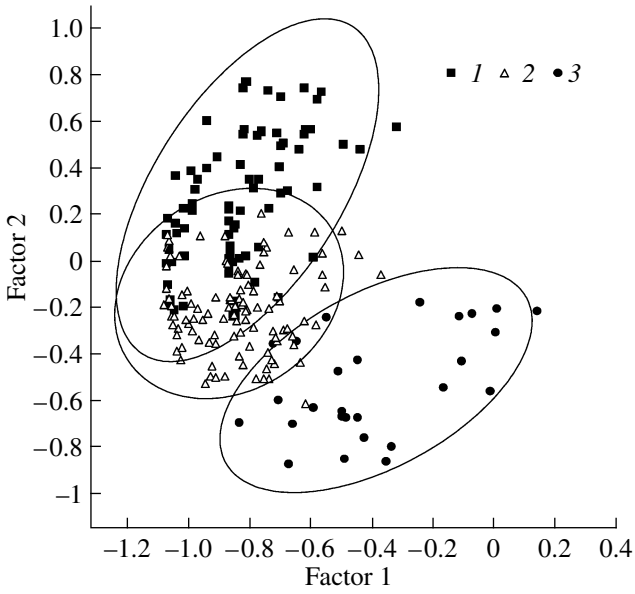

Fig. 3. PCA ordination of all analyzed Sorbus fruits according to the structure of the yeast population. Regions: (*1*) Moldova, (*2*) Moscow and the Moscow region, (*3*) Tver region.

lated. The results were the following: average similarity between groups on single fruits naturally decreased with increasing distance (Fig. 2). As expected, yeast groups on fruits collected in different regions that represent different natural zones are varied greatly. This observation is supported by ordination of yeast communities' structures of different fruits obtained using principal components analysis (PCA). Evidently, they group basically in geographical regions. The greatest differences are observed between the samples collected in Moldova and Tver region; Moscow and the Moscow region occupy an intermediate position.

Not only stochastic but also quite qualitative differences in yeast groups character of different regions were found. Thus, the representatives of *C. capitatum*, which regularly were observed on fruits collected in Moscow, the Moscow region, and Tver region, were never found in Moldova. In each region and in each geographical point, a natural increase in differences between groups with regard to distance is also observed. Yeast groups are most similar on fruits of single corymbs (77%), a bit less between different corymbs (60%), land even less on different trees in one point (55%).

The results demonstrate that the abundance and structure of single yeast groups are defined not only by a set of ecological factors (such as temperature, humidity, structure of environment), but also depend on the location and proximity to other groups. Thus, yeast communities on similar substrate resemble the other, when they are located close to each other. This law is demonstrated on various scales: from hundreds of kilometers in the case of different geographical regions to several meters in the case of fruits on one tree.

DISCUSSION

The spatial structure that causes high autocorrelation of synecological indicators is one of the prominent features of natural communities. As a rule, detection of species in a sample means a high probability of its detection in the neighboring samples. Obviously, this circumstance should be considered at a statistical assessment of various factors of the ecological community structure (Levin, 1992; Legendre, 1993). The aggregated character of population distribution in natural communities is caused by two types of phenomena: on the one hand, heterogeneity of distribution inhabitancy properties, on the another, features of reproduction and dispersion of organisms, such as clonality, colonialism, migration, etc.

The characteristics of the spatial structure of microbial communities have not been studied much. There are few mentions of this phenomenon in the literature. It has been demonstrated that, as a rule, horizontal distribution of soil microorganisms is not casual, but strongly aggregated in different scales: from millimeters to hundreds of meters (Ettema and Wardle, 2002). Statistical comparisons of the structure of soil microbial communities in topsoil using fractionation of total DNA (Franklin et al., 2002) revealed their strong structure. Thus, the genotypical similarity average between samples naturally decreased with increasing distance from several centimeters to tens of meters. The structure of bacterial fresh-water planktonic communities,

revealed with T-RFLP technique, was also especially similar, when points of sample aggregation were closely located. On average, the samples selected for the distance of 10 or 100 m and in various lakes differed ny 13, 17, and 75%, respectively (Yannarell, Triplett, 2004). Application of geostatistical methods allowed to reveal a strong aggregation of bacterial communities on a scale to 1 m in salted marchantia soils (Franklin, Mills, 2003).

Such studies demonstrate that the autocorrelation phenomenon is a characteristic feature for microbial communities. We should note that all authors of the mentioned works explain the observations by exclusively nonuniform distribution of environmental properties. In the case of soil or water habitats, autocorrelation of microbial parameters is really caused by the aggregated distribution of the environment. However, as was demonstrated in the present study, aggregation is also a characteristic of epiphytic microbial aggregations, formed in sufficiently unified habitats, such as fruits with high concentration of simple sugars. Fruit properties, compounds available to yeast, in particular, are basically defined by plant genome and the stage of its ontogeny. Nevertheless, yeast communities on fruits of one development stage turn out to be more similar when they are located closer to eachnother. So, distinctions in the yeast community structure in different geographical regions can be explained by differences in the conditions of their formation. The similarity of neighboring groups of fruit and on neighboring trees depends cell migration and cross-cotamination of fruits with yeast cells. The authors who studied the character of the distribution number of epiphytic bacteria on orange tree leaves (Lindow, Andersen, 1996) came to the same conclusions about the value of migration in the formation epiphytic microbial communities. They revealed that the neighboring trees have similarities such as the number of bacteria.

It should be noted that earlier we marked evident development of such yeast clonality. During long-term studies of the yeast population of various natural habitats, we repeatedly found that the specific structure of the yeast communities formed in similar or ecologically contrasting habitats appears especially alike when they are located close to each other (Maximova, 2000 settle down; Yurkov, Chernov, 2005). So, completely identical yeast strains, that do not differ in any morpholophysiological signs or PCR profiles, and obviously, representing clones, are usually allocated from samples selected in one geographical point (Wuczkowski, Prillinger, 2004; Inácio et al., 2005). While researching epiphytic yeast groups in the tundra and desert, which are characterized by extremely rarefied vegetative cover, we repeatedly found a lager number of phylloplane yeasts in the areas characterized by higher foliage cover (Chernov, etc., 1997). Evidently, the mechanism of these phenomena relies on cross-contamination mechanism. The major role is given to the factor of spatial proximity and probability of cell drift on a substratum, but not to the similarity of environmental conditions.

Evidently, propagation through cell transfer should play an important role in formation of microbial groups on accessible substrata during a limited period of time, such as juicy fruits, flower nectar, animal excrement, etc. In works on yeast ecology, it was suggested that contamination plays the initial role in formation of the specific structure of yeast in such communities, in particular, directed phoretic transportation of yeast cells to invertebrates (Babeva, Gorin, 1973; Starmer et al., 1988). However, the statistical acknowledgement of such mutual contamination was obtained for the first time.

Aggregation in the distribution of species of microorganisms, caused by migration and clonal colonization, should be taken into consideration in statistical assessments of their variety, for example, in monitoring, bioresource, and screening studies. During application of widespread factorial analysis, the presence of positive autocorrelation leads to understating intergroup dispersion and artificially overestimating intergroup variability (Maksimova, Chernov, 2004). The scale knowledge that demonstrates aggregation of microbial communities makes it possible to avoid such errors.

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