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Yeasts and Yeast-like Organisms Occurring in the River Morava

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Summary

One hundred and sixty seven yeast strains belonging to 27 species were isolated from 57 water samples of the river Morava of the Slovak Republic taken during one year. Sporobolomyces roseus, Candida maltosa, and Aureobasidium pullulans were found to be the most frequent species, from nearly 50% of all samples, and together with Saccharomyces cerevisiae, Rhodotorula glutinis, Cystofilobasidium capitatum, and Cryptococcus laurentii represented more than 80% of the total yeast population. Yeast densities of the river water ranged from 100–37,800 CFU per litre. The highest yeast population density was observed in samples taken in April, when Sacch. cerevisiae, Cr. laurentii and Cys. capitatum occurred the most frequently. The yeast community was characterized by a broad assimilation activity, with the xylose, cellobiose, and trehalose, which are widespread in nature, being assimilated by nearly 90% of population.

Keywords: river water, seasonal occurrence, ascomycetous and basidiomycetous yeasts

Introduction

Mycological examination of different water sources indicates that yeasts are present in all aquatic systems *(1–3)*. The number and species depend on the type and purity of water. The occurrence and distribution of yeasts in this environment was studied mostly in connection with water pollution *(4–6)*. The need for detection and enumeration of yeast-like fungi which are able to biodegrade and/or accumulate organic and inorganic toxicants has became greater as water pollution has increased *(7)*. The highly polluted areas of industrial countries could be home to microorganisms with the ability to degrade toxic wastes and survive in hostile environments, making them strong candidates for biotechnological discoveries *(8)*. Our previous studies gave the numbers and types of yeasts and yeast-like organisms found in stagnant water of artificial lakes and fish ponds *(9,10)*. There are few studies on the presence of yeasts in river water *(11–14)*. This paper deals with quantitative and qualitative studies of yeasts and yeast-like organisms isolated from the river Morava water of the Slovak section.

Material and Methods

The Morava river originates in Czech Republic and flows through the Slovak Republic to the river Danube. The Morava water is affected by the domestic and industrial wastes as well as agricultural run-off. Water samples were taken from seven sites within the lower stream of the river Morava. Grass, plants, broad-leaved trees and shrubs covered the river banks. Eight collections from each sampling point were made from May 1995 to May 1996. Water was collected in sterile bottles which were lowered into the river to a depth of 20 cm, transported to the laboratory on ice, and processed within of 2 hours after collection.

Aliquots of 10 mL water were precipitated with a colloidal solution containing 0.5 mL 10% sodium carbonate and 0.25 mL 10% ferric sulphate. After the centrifugation three drops of 20% sodium-potassium tartarate were added and the mixtures were spread onto malt agar plates containing chloramphenicol (Spofa) and incubated from 3 to 7 days at both 12 and 25 °C. Colonies

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of different appearance were counted and their representatives were purified according to Slávikova *et al. (9)*. The numbers of yeast colonies on plates were multiplied by 100 in order to obtain the CFU per litre.

Morphological and physiological characteristics of isolates were examined by the methods described by van der Walt and Yarrow *(15)*. Strains were identified according to Kreger-van Rij *(16)* and Kocková-Kratochvílová *(17)*.

Results

The temperature of the water ranged from 4 to 25 °C and pH reached a value between 6.92 and 8.72. One hundred and sixty seven yeast strains belonging to 27 species were isolated from 57 water samples of the river Morava (Table 1). *Sporobolomyces roseus*, *Candida maltosa*, and *Aureobasidium pullulans* were the most frequently isolated species being isolated from nearly 50% of all samples. Together with *Saccharomyces cerevisiae*, *Rhodotorula glutinis*, *Cystofilobasidium capitatum*, and *Cryptococcus laurentii* they formed more than 80% of the total yeast population. *Cryptococcus albidus* was present in 19% of the samples, but in a smaller number than *Cr. laurentii*.

Some species, *e.g. Geotrichum candidum* and *Candida lambica*, occurred frequently, but their contribution to the yeast counts of the river water was low (1% of the yeast counts). Thirteen species (*Candida bimundalis*, *C. guilliermondii*, *C. intermedia*, *C. parapsilosis*, *C. pulcherrima*, *C. sake*, *C. valida*, *Hansenula anomala*, *Pichia kluyveri*, *Rhodotorula minuta*, *Torulaspora delbrueckii*, *Trichosporon cutaneum*, and *Williopsis californica*) were found in one or two samples.

Table 1. The occurrence of yeast and yeast-like species isolated from the river Morava

Species	Frequency of isolation % (n = 57)	Total yeast counts % (n = 323 500 CFU/L)
Aureobasidium pullulans	42	31.0
Candida bimundalis	2	0.2
C. famata	7	0.6
C. guilliermondii	2	7.4
C. intermedia	2	0.03
C. krusei	9	0.4
C. lambica	16	0.6
C. maltosa	42	9.2
C. parapsilosis	2	0.3
C. pulcherrima	3	0.2
C. sake	2	0.2
C. tropicalis	7	1.7
C. valida	2	0.6
Cryptococcus albidus	19	3.6
Cr. laurentii	16	6.0
Cystofilobasidium capitatum	23	6.4
Geotrichum candidum	19	0.4
Hansenula anomala	2	0.06
Pichia kluyveri	2	0.7
Rhodotorula glutinis	12	7.8
Rh. minuta	2	0.2
Rh. rubra	7	1.05
Saccharomyces cerevisiae	14	9.3
Sporobolomyces roseus	47	11.7
Torulaspora delbrueckii	2	0.3
Trichosporon cutaneum	2	0.03
Williopsis californica	2	0.12

Yeast densities of the river Morava water ranged from 100 to 37,800 CFU per litre in the water samples. The average number reached approximately 5,700 CFU per litre. The highest yeast population density was observed in samples taken in April (the average density was 13,400 CFU per litre.). *Sacch. cerevisiae*, *Cr. laurentii*, *Rh. glutinis*, and *Cys. capitatum* formed the major part of the yeast population in that month (Figs. 1, 2). Surprisingly, the species producing carotenoid or melanin pigments made up around 60% of the yeast counts. A wide range of *Candida sp.* was found there. This genus was represented by twelve species, but only *C. maltosa* and *C. lambica* were isolated from more than 10% of samples.

The yeast community isolated from the Morava consisted predominantly of species with the ability to assimilate xylose, arabinose, cellobiose, trehalose, and soluble starch (Table 2). The fermentation of glucose was positive only for 31% of isolates, and two thirds of the yeasts and yeast-like organisms belonged to basidiomycetous species or anamorphs.

Table 2. Survey of some features of yeast population

Feature	Occurrence of yeast population with individual feature / %
Presence of urease	68
Fermentation of D-glucose	31
Assimilation of nitrate	61
Assimilation of D-xylose	90
Assimilation of L-arabinose	67
Assimilation of cellobiose	88
Assimilation of trehalose	88
Assimilation of soluble starch	69

Discussion

27 yeast and yeast-like species were isolated from the river Morava, but only eight or ten of them could be considered to be the prevalent. Many of these species occur in association with plants and soil.

The ubiquitous »black yeast« *A. pullulans* was one of the three most frequent species, and has been found in many types of water *(7,18)*, and it was among the prevalent species in artificial lake and fish-pond waters *(9,10)*. Normally it is associated with foliage and soil and probably enters the water with run-off. Its wide adaptation to diverse ecological conditions is discussed by Mukerji and Gupta *(19)* and it probably plays an important role in certain ecosystems. The occurrence of *A. pullulans* increased in spring and in May together with *Sp. roseus* made up 98% of the yeast counts (Fig. 1). This high number could be influenced by a storm occurring one day before our sampling. This should have flushed many microorganisms into the water from plants and soil. One year later the total yeast population during May sampling was lower (Fig. 1), but the species *A. pullulans* and *Sp. roseus* represented 73% of it.

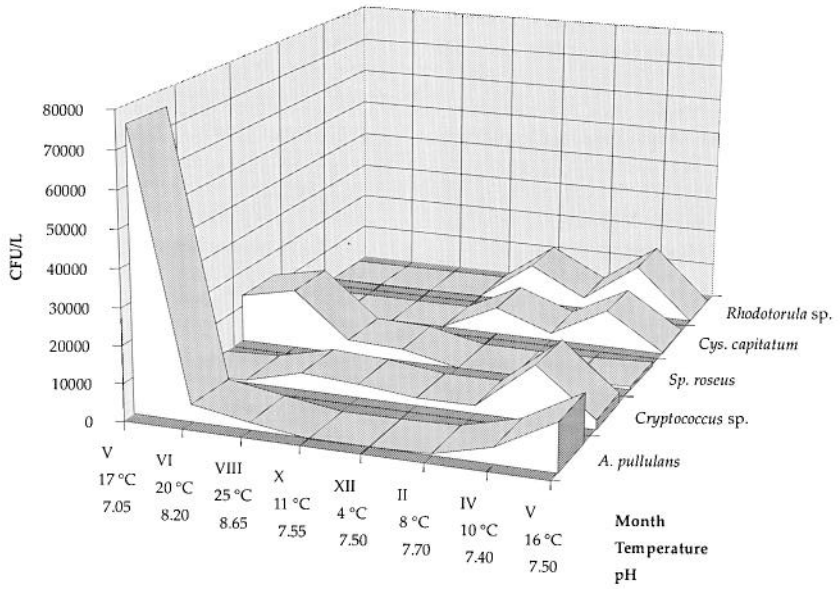

Fig. 1. Changes in concentration of the most frequently isolated black yeast and basidiomycetous yeast anamorphs during one year.

Sp. roseus is a species, which produces high level orange to red carotenoids, forms ballistoconidia, and is often associated with the phyllosphere of plants. During periods of bright sunlight, carotenoids protect the photosynthetic apparatus of plants against photodestruction *(20)*. Similarly, they may also protect the vital structures and processes of yeast cells. This is a possible reason for the predominance of red yeasts in the upper layers of water *(7,21)*. *Sp. roseus* was found in 47% of samples taken from the river Morava, and was a prevalent yeast in fish-pond water of the same region *(10)*. Some species of the genus *Sporobolomyces* were also isolated from seawater samples collected in the Pacific Ocean *(22)*.

Other carotenoids producing species, *Cys. capitatum* and *Rhodotorula* sp., were also regularly isolated organisms, with a large proportion of the isolates in December and April (Fig. 1). The occurrence of these species could also be affected by their ability to grow at lower temperatures. Nearly all strains of these species grew well at 5 °C. Most of the many red yeasts were identified as *Rh. glutinis*, a species frequently isolated from many types of water *(2,11,23)*. The »pink yeasts« even represented 58% of the total yeast isolates collected from the St. Lawrence River *(12)*.

C. maltosa occurred in higher proportion from October to February (Fig. 2). It was present in 42% of the samples and formed 9.2% of the total yeast population. *C. krusei*, *C. lambica*, and *C. tropicalis* were found less frequently. The large increases in the proportion of *Candida* yeasts in the environment are often associated with the presence of human wastes *(5)*. Some *Candida* spp. are characteristic of sewage and polluted water *(4,23)*.

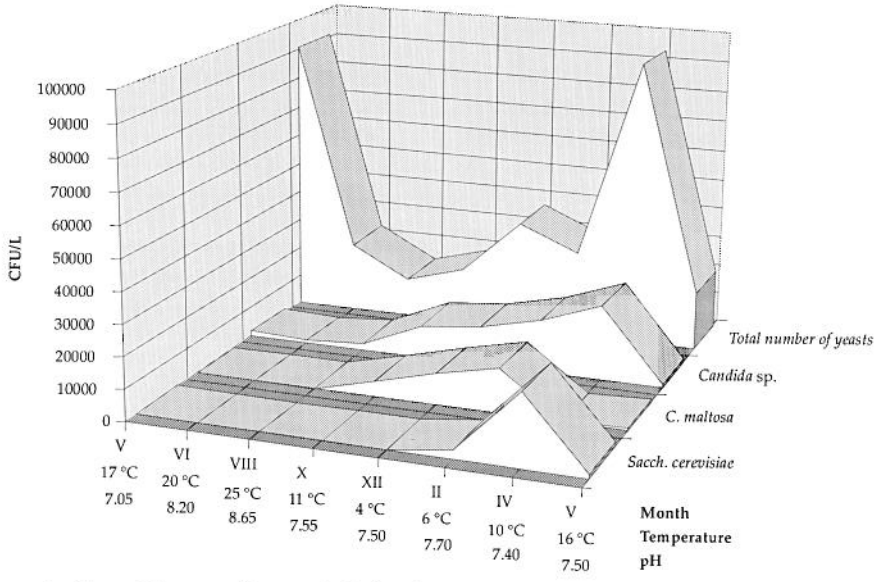

Fig. 2. Changes in concentration of the most frequently isolated ascomycetous yeasts and their anamorphs during one year

The genus *Cryptococcus* was represented by the species *Cr. albidus* and *Cr. laurentii*. These two species made up around 10% of the yeast counts found in the river Morava. They are prevalent in many aquatic environments *(2,11,23,24)*, and are often associated with plants and soils. Species of the genera *Candida* and *Cryptococcus* existed in water samples during the whole year, but their counts were the highest in April. The concentration of the total yeast population was the highest also in that month, presumably influenced by snow-melt in that season.

Sacch. cerevisiae appeared to be the only common ascosporogenous yeast species in the river Morava. We recorded its incidence from February to May (Fig. 2). It was reported that *Sacch. cerevisiae* was a stable constituent of activated sludge biocenosis of different industrial waste-waters *(25)*. Spencer *et al. (26)* isolated this yeast together with *Trichosporon cutaneum* as a dominant species in domestic sewage treatment plant; some strains were found in river water, too *(27)*.

We observed that yeast number in the river Morava was the lowest in August (Fig. 2). On the other hand, the mean pH of water (8.65) and the temperature of water (25 °C) in that month were the highest. The level of the river was very low at the same time and it seems that the quantity of precipitation, in that season minimal, very much influenced quantitative occurrence of yeast population.

Many additional factors might affect the number of yeasts in water environment, and determine the species that exist there. The presence of higher amounts of nitrogen and phosphorus, type of pollution and the ability of organisms to adapt to this surrounding may have the key influence. Decomposing woody material in the river along the bank was available for colonization by microorganisms. This fact undoubtedly contributed to the high participation of strains with the ability to utilize pentoses in water samples.

It is known that yeast species, frequently found in the river Morava, have various degradative abilities. The red yeasts, *Cr. laurentii*, and some *Candida* spp. degraded some phenolic or chlorophenolic compounds *(28–30)*. *C. maltosa* and *C. tropicalis* oxidized polycyclic aromatic hydrocarbons *(31)*. *Sacch. cerevisiae* showed ability to metabolize naphtalene and benzo(a)pyrene *(31)* and to bioreduce and remove heavy metals *(32,33)*. The strains of this species were used extensively in the microbial biotransformation *(34,35)*.

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Kvasci i kvascima slični organizmi u Moravi (Slovačka)

Sažetak

Stošezdesetsedam vrstâ kvasaca, pripadnika 27 sojeva, izolirano je iz 57 uzoraka vode iz Morave (Slovačka Republika) tijekom jedne godine. U približno 50% uzoraka najčešći su bili sojevi Sporobolomyces roseus, Candida maltosa i Aureobasidium pullulans, a zajedno sa Saccharomyces cerevisiae, Rhodotorula glutinis, Cystofilobasidium capitatum i Cryptococcus laurentii činili su više od 80% ukupne populacije kvasaca. Brojnost kvasaca u riječnoj vodi iznosila je od 100 do 38.800 CFU/L. U uzorcima vode uzete iz travnja najbrojniji su bili kvasci, a među njima Sacch. cerevisiae, Cr. laurentii i Cys. capitatum. Ta zajednica kvasaca odlikovala se velikom sposobnošću asimilacije, a spojeve rasprostranjene u prirodi (ksiloza, celobioza i trehaloza) asimiliralo je približno 90% populacije.