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Original papers

Share of the *Saccharomyces* genus in the mycobiota of the gastrointestinal tract of oncology patients – potential effects of a fruit-based diet

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ABSTRACT. This paper concerns the determination of the share of fungi of the *Saccharomyces* genus in the mycobiota of the gastrointestinal tract of patients with colorectal cancer. The biological material were fungi isolated from the mouth, oesophagus, duodenum, stomach, large intestine and anus of 92 patients with colorectal cancer. They were subjected to standard mycological diagnostic testing. The analysis was only carried out on isolates containing fungi of the *Saccharomyces* genus. In 140 isolates (24.5% of all positive results), the species *S. cerevisiae* (68.5%), *S. fragilis* (5.71%), *S. italicus* (11.43%), *S. ludwigii* (8.60%) and *S. rouxii* (5.76%) were found, which occurred individually or were accompanied species of the *Candida, Trichosporon*, and *Rhodotorula* genera. Most isolates were obtained from the large intestine and anus, and the least from the stomach. It follows from the comparison of the obtained results with those of previous studies into the occurrence of fungi of the *Saccharomyces* genus in various segments of the gastrointestinal tract, that their prevalence in the gastrointestinal tract is gradually increasing.

Key words: *Saccharomyces*, potential pathogenicity, gastrointestinal tract

Introduction

Owing to the development of interdisciplinary research, in the context of searching for the causes of the spread of surface and organ fungal infections of the yeast etiology [1], information on fungi commonly found in the human environment as opportunistic saprobionts is becoming increasingly important [2]. These include fungi of the *Saccharomyces* genus, which as early as several decades ago [3] were classified as potential human pathogens although separate studies were rarely devoted to them [4,5]. The most important natural reservoir of these fungi is the surface of fruit and vegetables, plant secretions and substrates rich in sugars and other organic compounds [6]. This does not exclude their asymptomatic occurrence in the human ontosphere: in the mouth and gastrointestinal tract, in the respiratory system or on the skin, particularly at sites with increased humidity [7]. This occurrence may be temporary but most frequently it is associated with physiologically-determined carrier status.

In a healthy individual, yeasts (along with bacteria) constitute the natural microbiota of the intestines in which adequate humidity, abundance of food and a constant temperature provide perfect conditions for existence. As commensals, they co-participate along with other microorganisms in the utilisation of food residues without causing harm to the host, and the constant trophic competition maintains *inter alia* balance.

In oncology patients, who are a group under particular risk of fungal infections, the general biological balance becomes disturbed in favour of yeasts. The rate of proliferation, count and expansiveness of yeasts which are able to move throughout the entire ontosphere increases in proportion to the degree of disturbance to this balance (the stage of a cancer, neutropenia, therapy).

Table 1. The number of fungal isolates of the *Saccharomyces* genus, obtained from the gastrointestinal tract

	Number of isolates/ontocenoses						
Type	mouth	esophagus	stomach	duodenum	colorectal	anus	Comments
Saccharomyces cerevisiae	26/8*	7/1*	0	9/4*	40/7*	25/13*	*C. albicans*, *C. dubliniensis*, *T. beigelii*, *C. krusei*, *C. glabrata*, *C. guilliermondii*, *C. tropicalis*
Saccharomyces italicus	0	3*	5*	0	0	8*	*C. albicans*, *C. dubliniensis*, *T. beigelii*, *C. krusei*, *C. glabrata, C. guilliermondii*, *Rhodotorula* spp.
Saccharomyces ludwigii	4*	4*	4*	0	0	0	*C. albicans*, *C. guilliermondii*, *Rhodotorula* spp.
Saccharomyces fragilis	4	0	0	4	0	0	*Rhodotorula* spp.
Saccharomyces rouxi	4	4	0	0	0	0	

*multi-species isolates

The list of factors predisposing to fungal infections includes a diet rich in carbohydrates, particularly in simple sugars, which are the main source of carbon for yeasts. Taking into account the general biological determinants in oncology patients, which stimulate the development of fungi and food preferences of yeasts, a question arises as to whether a fruit-based diet is recommended for patients with cancer.

Materials and Methods

The biological material (swabs, intraoperative specimens, endoscopy) collected by clinicians was fungi isolated from the mouth, oesophagus, duodenum, stomach, large intestine and anus of 92 patients with colorectal cancer. The material was sampled for general mycological testing assessing the taxonomic diversity, prevalence and the degree of colonisation of these patients' ontosphere by various fungi [1].

Biological materials were subjected to standard diagnostic testing recommended by mycology laboratories [8]. Starting cultures were maintained on a liquid Sabouraud medium with the addition of gentamicin (0.025%) and chloramphenicol (0.1%) for 7–14 days at temperatures of 37°C and 25°C until the fungi had grown. The material was then sieved onto a solid Sabouraud medium with the same antibiotics and incubated for 48 hours at a temperature of 37°C. From the obtained strains, microcultures were established on Nickerson agar enriched with broth and serum in a 1:1 ratio. Incubation was carried out for 48 to 72 hours at 37°C. Samples with yeasts were also evaluated for their ability to ferment and assimilate sugars and the formation of asci on Gorodkova agar and of pseudohyphae on a medium with maize flour was observed. For the identification of taxonomic composition, keys by Barnett, Payne and Yarrow [9], De Hoog, Guarro, Gene and Figueras [10] Kurtzman and Fell [11] and Kurtzman, Fell and Boekhout [12] were used.

Results

A total of 570 isolates of fungi occurring at the same time in several ontocenoses of the same patient were obtained from the examined patients. In 140 isolates (24.5%), five species of the *Saccharomyces* genus, i.e. *S. cerevisiae*, *S. fragilis*, *S. italicus*, *S. ludwigii* and *S. rouxii*, were found. The dominant species was *S. cerevisiae* (68.5%). The fungi were noted individually (72 isolates=51.4%) or together with other fungi (68 isolates=48.6%); most frequently they accompanied species of the *Candida*, *Trichosporon* and *Rhodotorula* genera (*Candida albicans*, *C. dubliniensis*, *C. guilliermondii*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *Trichosporon beigelii* and *Rhodotorula* spp.). Most yeasts were isolated from the large intestine (40) and anus (33), and slightly less from the mouth (29). The number of isolates in the oesophagus and duodenum was similar and amounted to 17 and 12, respectively. On the other hand, only nine fungi isolates were noted in the stomach (Table 1).

Discussion

Multi-annual analyses of biological materials

originating from oncology patients indicate an evident increase in the prevalence of yeasts of various taxa in this group of patients [1,13,14]. The displacement of species previously regarded as dominant and, to a large extent, responsible for fungal infections, by species regarded as unimportant for clinical reasons, has been increasingly observed [2,7]. Most often this concerns the displacement of *C. albicans* by other species of this genus, e.g. *C. dubliniensis*, *C. glabrata* or *C. krusei*, or by species belonging to other genera e.g. by *T. beigelii* or *Rhodotorula rubra* [2]. It appears that this group of fungi which are increasingly often colonising ontocenoses of the gastrointestinal tract, particularly in oncology patients, includes fungi of the *Saccharomyces* genus as well. This is demonstrated by the results obtained over a period of several years. In the years 1991–2000, the share of *S. cerevisiae* in the mycobiota of the gastrointestinal tract of oncology patients with cancer was 4.5% [7]. In the period of 1999–2004, it increased to 11% [1,14], and in the years 2013–2016 to 12%; moreover, new species emerged, namely *S. fragilis* and *S. ludwigii* [14]. Currently, fungi of the *Saccharomyces* genus account for up to 24.5% of all positive results which allows them to be classified as dominants of the gastrointestinal tract mycobiota. All of them may enter the ontosphere via the mouth as contaminants of food products with the addition of fruit, or on the surface of fruit, particularly those with a high sugar content.

It should be stressed that, in accordance with the basic ecological principle, a species colonising a new ecological niche is, as a rule, more expansive than those which settled in it earlier. Therefore, it is necessary to very closely observe all fungi whose prevalence in the ontosphere is increasing, particularly in the gastrointestinal tract, which is the most frequent route of the intrasystemic transmission of fungi. The aggressive spread of fungi, as well as the changed affinity for macroorganism's organs are frequently noted. Fungal cells move to the oesophagus, stomach, duodenum and intestines [7,15,16], and, in extreme cases, they may infiltrate the oesophagus and spread through the blood to the liver and cardiac valves [5,17,18]. Fungi of the *Saccharomyces* genus may colonise the mucous membranes of various organs. They are mentioned as the cause of nosocomial infections in immunosuppressed patients hospitalised for a long time and of infections of the urogenital system, post-operative and decubital wounds [4,19] as well as of fungal infections following an antibiotic therapy, dental procedures, and while using cardiac drugs [10,14]. Cases of fungemia involving *S. cerevisiae* and *S. fragilis* have been reported. The fact that seems to be very important is the actual presence of fungi in several ontocenoses of the gastrointestinal tract of the patient. It was proven that multifocal colonisation of the gastrointestinal tract by fungi creates a greater risk of spreading the invasion through the blood to other internal organs than their settling in only one ontocenosis [5,14,19]. The identification of the presence of fungi in the mouth is very often accompanied by their detection in more distant segments of the gastrointestinal tract. On the other hand, the isolation of fungi from the anus suggests that the invasion centre is located in the large intestine [14], which closely corresponds to the results obtained from the examined patients.

Of the potential pathogenic species of the *Saccharomyces* genus, *S. cerevisiae* is the most frequently mentioned [3–5,10,14]. Currently, *S. italicus* has also been included in this species due to the ability to ferment sugars [9,19,20]. However, for practical reasons, *S. italicus* was indicated in this study as a separate species which most frequently settles on citrus fruits [20]. *S. ludwigii* was included into the *Saccharomycodes* genus as *S. ludwigii*, and *S. fragilis* to the *Kluyveromyces* genus as *K. marxianus* [9]. The anamorphic form of the latter is *Candida kefir,* a species much more often mentioned as an opportunistic fungal pathogen in immunosuppressed patients [9,18]. The only species whose clinical significance was not determined is *S. rouxii* (currently *Z. rouxii)* [9].

The entry of fungal infections most often leads through the villi of the small intestine, through which the fungi are translocated from their site of existence, which results in an endogenous infection [14,15,19,21]. An exogenous infection originating, in the case of the *Saccharomyces* genus, from fruit is also possible. Therefore the authors posed the question in the introduction.

It was found that these yeasts accompany, as a rule, other fungi which are frequently species with high pathogenicity, which is also confirmed by the results. They are believed to be the direct cause of lesions, and fungi of the *Saccharomyces* genus are only a physiological component of this [22,23]. However, an opposite situation cannot be ruled out, especially since the expansiveness of fungi in

relation to the human ontosphere is increasing and the number of factors predisposing to fungal infections is increasing as well [1,7,14].

The surface of the fruit is a rich reservoir of yeast of the *Saccharomyces* genus, whose expansion in relation to the human ontosphere and prevalence in the gastrointestinal tract is gradually increasing. These fungi included in the diet of oncological patients may have a potential impact on the appearance of fungal yeast etiology.

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