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Significant Reduction of *Saccharomyces cerevisiae* in the Gut Microbiota of Colorectal Cancer Patients from Iran

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ABSTRACT

Backgrounds: This study aimed to investigate and compare the prevalence of *Saccharomyces cerevisiae* (*S. cerevisiae*) in the stool of colorectal cancer (CRC) patients and healthy controls.

Materials & Methods: Over a one-year study period from April 2022 to April 2023, 25 patients who met all the necessary conditions for inclusion in the study were selected from among the patients referring to the colonoscopy unit of a teaching hospital in Tehran, considering the exclusion criteria. Stool samples were collected and immediately transported to the laboratory in a cold chain. Also, 25 healthy individuals who were matched with the patients in terms of sex, age, and ethnicity were included as a control group. After DNA extraction, the presence of *S. cerevisiae* was assayed by TaqMan real-time polymerase chain reaction (PCR) using specific primers and probe.

Findings: The cancer stage of all individuals in the CRC group was 3, and the location of tumor formation in all of them was colon. Of the 25 samples in each group, three samples in the CRC group and 18 samples in the healthy group were positive for the presence of *S. cerevisiae* DNA. This difference was statistically significant ($p < .0001$).

Conclusion: The results may indicate the importance of evaluating *S. cerevisiae* reduction as an indicator for the diagnosis of CRC. Considering the positive role of *S. cerevisiae* in maintaining gut health and also in helping treat CRC, it could be used as a probiotic product or adjuvant in this field.

Keywords: *Saccharomyces cerevisiae*, Colorectal cancer, Real-time Polymerase Chain Reaction

CITATION LINKS

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Introduction

Probiotics are living organisms that, if present in sufficient and certain amounts in the body, have numerous benefits for humans. These benefits include: controlling digestive infections, improving the breakdown or absorption of nutrients, exerting anti-cancer and anti-inflammatory effects, reducing blood lipids such as cholesterol, helping improve or prevent certain digestive disorders such as inflammatory bowel disease and irritable bowel syndrome, and stimulating and developing the immune system [1]. Probiotics include various types of bacteria as well as some fungi [2]. One of the fungi, which is referred to as a probiotic and has significant positive effects on gut health, is *Saccharomyces cerevisiae* (*S. cerevisiae*) [3] *S. cerevisiae* is a beneficial budding yeast that is present in many fermented foods (such as bread), some beverages, and thus the human digestive tract as a member of the gut microbiota [4]. In addition to its probiotic properties, this beneficial yeast specifically helps improve and develop gut immune responses and strengthen the intestinal barrier. It also has anti-inflammatory effects by increasing the production of Interleukin 10 (IL-10). It also helps improve diarrhea caused by *Clostridioides difficile*, which, if left untreated, could eventually lead to colon cancer [5]. In addition, *S. cerevisiae* also has anti-cancer effects on digestive cancers such as colorectal cancer (CRC) [6, 7].

CRC is the second leading cause of cancer-related death and the third most common cancer worldwide [8]. Several factors are involved in CRC formation and progression, but one of the factors that researchers have paid a lot of attention to, focused on, and researched, especially in the last decade, is the issue of changes in the type and abundance of the gut microbiota (dysbiosis). Dysbiosis could potentially lead to CRC through its effects on inflammatory processes as well as genetic and epigenetic changes [9]. *S. cerevisiae* helps prevent CRC and reduce postoperative complications of this cancer through various mechanisms such as inhibiting carcinogenic compounds, competing with pathogenic microbes, modulating immunity, and regulating apoptosis and cell differentiation [9, 10].

Objectives: However, research has shown that this beneficial yeast is significantly reduced in patients with CRC [7]. Given the importance of *S. cerevisiae* in maintaining intestinal health, the present study examined and compared the prevalence of this yeast in the colon contents of CRC patients and healthy individuals from Iran.

Materials and Methods

Characteristics of study participants and sample collection: Over a one-year study period from April 2022 to April 2023, all patients referring to the Colonoscopy Unit of Imam Khomeini hospital (Iran, Tehran) for colonoscopy were examined in terms of the present study inclusion and exclusion criteria. On admission, patients had symptoms such as constipation, diarrhea, anemia, blood in the stool, rectal bleeding, weight loss, and abdominal pain, aches, or cramps. Initial confirmation of CRC in these people was done by a specialist doctor based on colonoscopy results, and then final confirmation was performed by a pathologist after surgery and sending the tumor biopsy sample to the pathology laboratory.

After confirming the diagnosis of CRC, they were examined in terms of cancer staging. To determine the stage of CRC, different methods were used, including computed tomography, magnetic resonance imaging , and ultrasonography of internal organs; also, a blood test was performed to check serum carcinoembryonic antigen levels [11]. The cancer staging process was done following the tumor, node, metastasis [12] protocol published by the American Joint

Committee on Cancer [12] . The inclusion criteria for the CRC patient and control groups were the presence and absence of CRC, respectively. Members of the control group were selected from among people who underwent colonoscopy but had no signs or symptoms of polyps, cancerous tumors, or any inflammation or digestive disorders. Exclusion criteria for both groups included: having a body mass index (BMI)>30 kg/m^2, being a vegetarian, having severe malnutrition, having other gastrointestinal malignancies, using antibiotics and probiotic products in the past three months, using immune-modulatory drugs, and undergoing any therapeutic intervention such as chemotherapy, radiotherapy, and surgery [13]. According to the above criteria, 25 CRC patients and 25 healthy volunteers were included in the study as the patient and control (without CRC) groups, respectively. In order to increase the accuracy and precision of the results, the control group was matched with the patient group in three variables of age, sex, and ethnicity. Stool samples were collected from both groups before surgery or any therapeutic intervention in special stool sample collection containers. Stool samples were quickly transferred to the Bacteriology Laboratory of Tarbiat Modares University under cold chain conditions within a maximum of two hours from the time of receipt.

DNA extraction: The total DNA content of feces (500 mg) of all study subjects was extracted using the Favorgen Stool DNA Extraction Kit (Stool DNA Isolation mini Kit, Favorgen Biotech, Taiwan) based on the kit instructions. Agarose (1%) gel electrophoresis was performed to check the quality of the extraction process in terms of the existence of DNA fragments of appropriate size and the absence of very small fragments. The amount of DNA in each microliter of the extracted sample was measured with a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). All DNA specimens were maintained at -20 °C. The remaining fecal samples not used for DNA extraction were first divided into three containers and finally stored at -80 °C for later use. In this study, *S. cerevisiae* ATCC 18824 was employed as a control [14, 15].

TaqMan real-time polymerase chain reaction (PCR) assay: In order to identify *S. cerevisiae* in the stool samples of the two groups of CRC patients and healthy controls, TaqMan real-time PCR method was used. This method used specific primers and probe, schemed to bind to *S. cerevisiae* strains and identify this yeast in the samples (forward primer: 5-′GAAATGCCACCGTGAATGC-3′/ reverse primer: 5′-CTTTGGTGGTGATCCTCTATGATTG-3′/ probe: FAM-TGGCACCATGAACCCTAGCGTCGTT -TAMRA) [16]. The primers were examined for probable mismatches, specificity, and inclusivity using primer-BLAST web tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast) and SILVA high-quality ribosomal RNA database (https://www.arb-silva.de).

The primers were also examined for melting temperature and secondary structures using OligoAnalyzer tool (https://www.idtdna.com/pages/tools/oligoanalyzer). To overcome non-specific off-target amplifications, the annealing temperature was set at a few degrees below the melting temperature [17]. TaqMan real-time PCR was conducted on a Stratagene Mx3000 real-time PCR cycler (Agilent, US) using 2X SYBR Green real-time PCR master mix (Ampliqon, Denmark) to detect *S. cerevisiae*. Amplification reactions were carried out in a final volume of 20 µL. Reaction mixtures contained 10 µL of 2X SYBR Green real-time PCR master mix, 2 µL of template DNA, 1 µL of each of forward and reverse primers and probe, and 5 µL of ultrapure distilled water. TaqMan real-time PCR temperature program was as follows: an ini-

tial denaturation at 50 and 95 °C for 2 and 10 min, respectively, followed by 45 cycles that consisted of denaturation for 15 s at 95 °C, annealing for 1 min at 60 °C, and extension for 30 s at 70 °C [16]. Negative controls that contained all the reaction constituents except the template DNA were run in parallel with each amplification reaction, but no amplified DNA fragments were detected. At last, the samples melting temperature was recorded, and their amplification curve was plotted. *S. cerevisiae* ATCC 18824 DNA was employed as a control sample.

Statistical analysis: Data were analyzed using SPSS software Ver. 26.0 (SPSS Inc., Chicago, IL, USA). Chi-square test was employed to test the difference in the prevalence of *S. cerevisiae* in the stool of CRC patients and healthy controls. Statistical tests with $p < .05$ were considered significant.

Findings

Patient data and CRC staging process: This research was conducted on 25 CRC patients and 25 healthy controls. There were 13 males and 12 females in each group. In terms of age, nine people were under 50 years old, five were between 50 and 60 years old, and 11 were over 60 years old. All 25 members of the patient group had stage 3 CRC. The patients and healthy subjects were matched for age, sex, and ethnicity to increase the accuracy and precision of the results. General information about the members of both groups was as follows: their age range was between 28 and 68 years, all of them had a BMI < 30 kg/m^2 and between 22.2 and 29.2 kg/m^2 based on the exclusion criteria, and the tumor location of all members of the patient group was in the colon. In this study, the sample size was confirmed by referring to the book by Tabachnick and colleagues (2013) [18] to obtain reliable results.

Differences between healthy and CRC groups in the presence of *S. cerevisiae:* After analyzing the TaqMan real-time PCR plots, it was determined that three out of 25 samples in the CRC group and 18 out of 25 samples in the healthy group were positive for *S. cerevisiae*. By conducting the statistical test, it was determined that the above findings indicated a highly meaningful difference between the patient and healthy groups regarding the existence of *S. cerevisiae*

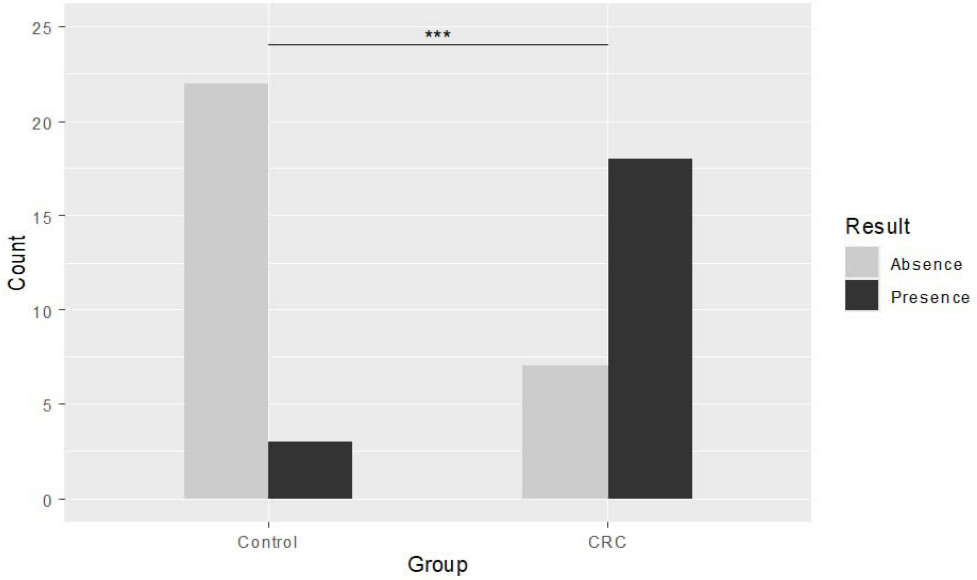

Figure 1) Significant difference in the prevalence of S. cerevisiae in the stool of colorectal cancer (CRC) patients and healthy controlls after statistical analysis

in the colon contents of these individuals ($p<$ .0001) (Figure 1).

Discussion

The fungal population in the gut is called the mycobiome. *S. cerevisiae* is the most abundant member of the human gut mycobiome, whose balance is very important for gut health compared to other fungi residing in the colon [19, 20]. This fungus, which is referred to as a probiotic, could help prevent or treat CRC, in addition to its beneficial effects on maintaining and promoting intestinal health [21].

Researchers have investigated how *S. cerevisiae* affects CRC. According to the study by Sambrani et al. (2019), the anticancer effect of *S. cerevisiae* is mediated by increasing apoptosis of cancer cells and reducing their metastasis [21]. Lee et al. (2005) also found that this yeast inhibited the growth and survival of CRC cells [22]. Some strains of *S. cerevisiae* are the main producers of short-chain fatty acids in the gut, and one of the most important effects of these compounds is their anti-inflammatory and anti-cancer properties [9]. In general, *S. cerevisiae* is an indicator of gut health and is more prevalent in the stool of healthy people compared to those with digestive diseases [23]. Among the fungi that naturally live in the human intestine, *S. cerevisiae* has the most positive effects, and negative effects have rarely been reported. For example, Algazaq et al. (2017) showed that this yeast could lead to opportunistic infections such as fungemia in patients with immunodeficiency [24]. On the other hand, many studies, such as the study by Ka´zmierczak-Siedlecka et al. (2020), have indicated that the prevalence of *S. cerevisiae* in the colon content of CRC patients is significantly reduced compared to healthy people [25]. Given the positive role of this yeast, especially in increasing IL-10 production and reducing tumor necrosis factor-alpha secretion, and consequently, considering its anti-inflammatory properties, its reduction in the colon content of CRC patients would not have a good prognosis [26]. The significant reduction in the prevalence of fungi detected in feces, particularly *S. cerevisiae,* is so important that recent research has suggested it could be used as a biomarker in the diagnosis of CRC [27]. In the current research, the number of *S. cerevisiae*-positive samples was substantially higher in the healthy group compared to the CRC group; in fact, the patient group showed a significant reduction in the prevalence of this yeast. As mentioned earlier, most other studies such as Wang et al. (2023) and Ilive and Leonardi (2017), similar to the present study, have reported a sharp decrease in the level of *S. cerevisiae* in the colon content of CRC patients [28-30]. Although the results of this study are statistically reliable, it would be better to use larger sample sizes in similar studies in the future, for example, with the help of multicenter collaborations, etc.

Conclusion

In this study, an attempt was made to prevent the role of confounding factors as much as possible by considering strict exclusion criteria, but the role of lifestyle such as activity, diet, stress, etc. in the composition of the colon microbiota could not be ignored. However, the results of TaqMan real-time PCR in the present study, which was used for the first time in Iran to identify *S. cerevisiae* in the colon contents of CRC patients, showed a very significant decrease in the prevalence of this fungus in the CRC group compared to the healthy group. This finding could be considered as another confirmation that strengthens the candidacy of this yeast as a diagnostic biomarker for this cancer; therefore, given the positive effects of *S. cerevisiae* on preventing CRC progression and reducing its complications, it could be used as a pro-

biotic product or adjuvant alongside other drugs to help improve CRC, necessitating the need for further and more detailed research. It is important to note that there are some points to consider when using this yeast as a probiotic product; for example, it seems that in patients with immune system deficiencies or people with cancer, excessive consumption of this yeast sometimes leads to fungemia, which could have devastating effects on the host.

Therefore, the *S. cerevisiae* probiotic product must be prescribed by a specialist physician and consumed under his supervision.

As a suggestion for future research, researchers could examine *S. cerevisiae* levels in the gut of CRC patients before and after treatment using multi-omics (metagenomics, metabolomics) approaches, they could also examine with greater precision not only the fungi of the gut microbial population but also the entire human microbiota.

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Ethical permissions: This research was approved by the Ethics Committee of Tarbiat Modares University (ethics code: IR.MODARES.REC.1399.083). In addition, a signed written consent form was obtained from all the studied subjects, indicating their awareness and agreement to conduct the present research on their stool samples.

Authors' contributions: B.B, MS.F, F.F: study idea, study design, work supervision. MS.F, T.Sh: sample collection. B.B, F.F, T.Sh: molecular techniques and manuscript writing/editing. A.R: data analysis. All authors read and approved the published version of the manuscript.

Conflict of interests: The authors declare that they no conflicts of interest.

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