Antifungal activity of lactobacillus present in the yogurt on candida albicans in vitro

Atividade antifúngica dos lacatobacillus presentes no iogurte sobre a candida albicans in vitro

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RESUMO

Objetivo: Este artigo tem como objetivo avaliar a atividade antifúngica in vitro dos Lactobacillus bulgaricus e Streptococcus thermophilus que estão presentes no iogurte sobre o crescimento da Candida albicans. **Método:** Foi realizado o repique da Candida albicans, fornecido em um tubo, na placa de petri contendo o ágar sabouraud dextrose para formação de colônias e aumento da população de Candida albicans. As amostras foram diluídas em soro fisiológico e semeada na placa de petri e incubou-se por duas horas. Após as duas horas, realizou-se a semeadura do iogurte, contendo os Lactobacillus bulgaricus e Streptococcus thermophilus, sobre a placa de petri contendo a Candida albicans e incubou-se na estufa a 37º C por 48 horas. **Resultado:** Após o período de incubação, verificamos que houve a inibição do crescimento da Candida albicans, reduzindo a quantidade de colônias das placas onde foi semeado o iogurte. **Conclusão:** Os Lactobacillus presentes no iogurte foram eficazes na atividade antifúngica contra a Candida albicans in vitro. Atualmente, poucos estudos foram realizados in vitro, sendo necessário realizar novos experimentos isolando os Lactobacillus bulgaricus e Streptococcus thermophilus presentes no iogurte, para maior certeza da eficácia da atividade antifúngica de cada Lactobacillus.

Descritores: Candida albicans; Lactobacillus bulgaricus; Streptococcus thermophilus; Iogurte.

ABSTRACT

Objective: this paper aims to assess the antifungal activity in vitro of Lactobacillus bulgaricus and Streptococcus thermophilus that exist in yogurts on the Candida albicans growing. **Method:** we ringed the Candida albicans, that was provided in a tube and in a Petri´s plate, containing agar Sabouraud dextrose to compose the colonies and increase the Candida albicans population. Samples were diluted in physiological solution and seeded in the Petri´s plate, being incubated for two hours. After this time, we put the yogurt- containing the Lactobacillus bulgaricus and the Streptococcus thermophilus on the Petri´s plate. It was incubated in the heating chamber under 37oC for 48 hours. **Results:** After the incubation period, we verified that Candida albicans growing was inhibited, reducing the number of colonies in plates where the yogurt was seeded in. **Conclusion:** the Lactobacillus existent in the yogurt has an effective antifungal activity in vitro. Nowadays, few investigations were conducted in vitro, being necessary another studies to ensure the antifungal activity effectiveness of Lactobacillus present in yogurts.

Descriptors: Candida albicans; Lactobacillus bulgaricus; Streptococcus thermophilus; Yogurt.

ORIGINAL

Introduction

Currently, fungal infections are an important and growing public health problem due to the number of immunocompromised patients and to the incidence that has gradually increased in recent decades. Consequently, the morbidity and mortality caused by these pathologies have presented high rates in the last years[1-2], and Candida albicans yeast is already the second most frequently isolated in Brazil[3] and the most frequent isolated in humans.[1]

Candida albicans is a yeast pathogenic to man, first observed in 1839 by the German surgeon Bernhard Rudolf Konrad von Langenbeck, being erroneously considered the cause of *typhus* since it was observed in mouth ulcers of a patient who had the disease. In 1842, this microorganism was classified by physician David Gruby in the genus *Sporotrichum* and defined oral candidiasis. This microorganism was studied in detail by Berg in 1846, definitively establishing its relation with oral candidiasis. Years later, in 1853, this microorganism was denominated by Chales Robin of *Oidium albicans* and redenominated by Zopf of *Monilia Albicans* in 1890. Only in 1923 this species was transferred to the genus Candida and later the species Candida albicans was created by Berkhout. Throughout history, more than 111 denominations have been attributed to this yeast.[1,4]

The Candida genus is the main group of yeasts that cause opportunistic infections[5]. This genus is composed of a certain number of 150 to 200 species[1,5-8], which normally live in body niches such as oropharynx, buccal cavity, gastrointestinal tract, urogenital system, respiratory tract mucosa, skin folds, bronchial secretions and vagina.[5-6] From the total number of opportunistic infections, 90% are caused by the species *Candida albicans* (which corresponds to 50% of the total value, being the most prevalent species), *Candida globrata*, *Candida parapsilosis* and *Candida tropicalis*.[2]

Candida spp. is taxonomically classified in the *Fungi* kingdom, *Eumycota* division, *Deuteromycotina* subdivision, class *Blastomycetes*, family *Cryptococcacea*.[1] It is a dimorphic fungus, which has the capacity to present in different morphologies, in yeast forms (associated with asymptomatic colonization), *pseudohyphia* or *true hyphae* (associated with pathogenic processes).[2, 6, 9]

Candida albicans yeasts become pathogenic in patients with immunodeficiency. In these circumstances, they can cause disease in various organs and tissues, causing superficial and systemic infection.[1-2,5] There are several causes that can lead to development, such as immunosuppressive drugs, prolonged hospital stay, hemodialysis, renal failure, diabetes, broad spectrum antibiotics, cancer, chemotherapy, as well as surgeries and transplants of organs or bone marrow.[2]

Candida albicans has basically three types of manifestations: cutaneous, cutaneous and systemic mucus. Cutaneous mucus candidiasis affects the oral cavity and the vaginal canal, which is the most common form in humans. Cutaneous candidiasis can affect the moist areas of the body, such as interdigital spaces, breast regions, underneath the nails, folds of the groin and armpits, and consist of superficial manifestations.[5,7] Systemic or invasive infections, known as candidemia, are deep infections that affect the

bloodstream or organs that are disseminated in the bloodstream1, and mortality, despite available treatments, is currently high, occurring in 15 to 25% in the case of adults and about 10 to 15% in children.[2]

Vulvovaginal candidiasis (CVV) is a disease characterized by infection of the vulva and vagina, caused by yeast infections of the genus Candida, which measure approximately 2 to 6µm.[4,7-8] *Candida ssp*. can also make up the normal flora of the vagina and gastrointestinal tract where, under normal conditions of the immune system, it does not cause any harm, only when it begins to proliferate too much causing candidiasis.[6,8] Vulvovaginal candidiasis affects at least once about 75% of women have different risk factors for the development of this form of disease, such as: use of hormones, antibiotic treatment, use of local contraceptives and diabetes.[2,10]

Oral candidiasis is the most common opportunistic infection in this cavity, which may present in different forms, such as acute pseudomembranous candidiasis, chronic atrophic candidiasis, angular cheilitis, leukoplakia and chronic cutaneous mucosal candidiasis.[2]

The effective therapeutic treatment for the treatment of Candida is composed of four groups of drugs that are polyene, triazolic, echinocandins and fluocitosin.[7] The treatment of oral and vaginal Candida, which aims to improve symptomatology, is performed through antifungal drugs[7-8,11], including those for oral and vulvovaginal candidiasis such as nystatin, clotrimazole, amphotericin, fluconazole, ketoconazole, butoconazole, clotrimazole, miconazole, nystatin, thioconazole, among others.[7]

For severe systemic cases, such as meningitis, treatment consists of the prescription of amphotericin-B. It is prescribed by the physician and administered at the hospital level, since it requires intravenous use and has high levels of nephrotoxicity.[7] In this sense, new studies indicate the medicine Ambisome (Amphotericin B liposomal), which contains the same active principle, has a special composition that causes less severe and less frequent adverse reactions. However, the indiscriminate use of drugs with inadequate doses and the increasing use of these drugs in the prophylaxis of fungal infections have increased the clinical resistance of *Candida albicans* to antifungal agents.[4]

Since probiotics have several beneficial effects on the balance of the intestinal flora, strengthening the immune system, which are aids in the treatment of vaginal and recurrent candidiasis.[12] Whereas, it was initially defined as compounds or extracts of tissues capable of stimulating microbial growth. In 1974, Parker defined probiotic as relating to organisms and substances that contributed to the intestinal microbial balance, however, "substance could include supplements such as antibiotics, which have an opposite function, hence they have abandoned this definition. By the 1990s, a more specific definition was given in which it defined probiotics as viable microorganisms, which includes lactic and yeast bacteria in the form of lyophilized cells or fermented product that have a beneficial effect on the health of the host after ingestion due to improvements in the properties of the native microflora.[13]

In addition, according to RDC Resolution No. 2 of January 7, 2002, probiotics are living microorganisms capable of improving the intestinal microbial balance producing beneficial effects on the health of the individual.

Thus, yogurt is a functional food because it is a source of calcium, but recent research has studied the probiotic effects of fermented dairy products14, produced by coagulation and acidification of the pH of the milk, or reconstituted milk, and may be added or not , from other dairy products through milk fermentation through the protossymbiotic action mechanism of L*actobacillus delbruckii subsp bulgaricu*s and *Streptococcus thermophilus*.[15]

Due these definitions and the need to develop alternative and/or complementary treatments that reduce the rate Candida albicans resistance to commercially available drugs, we aimed to assess the antifungal activity in vitro of Lactobacillus bulgaricus and Streptococcus thermophilus that exist in yogurts on the Candida albicans growing.

Method

This is an experimental study, carried out in the laboratory of a private college in the state of Goiás from April 27, 2019 to May 2, 2019.

For the experiment, a strain of *Candida albicans* and a sample of natural yoghurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, without sugar and Nestlé® preservatives, were used. Lot 9097132322, date of manufacture 07.04.2019 22:26, expiration date 22.05.2019.

The experiment was carried out using the Sabouraud dextrose agar medium - a medium for the isolation of yeasts and fungi prepared according to the European Pharmacopoeia formulation and the US Pharmacopoeia (Microbial Test of non-sterile products: Test for specific microorganisms).

After obtaining the sample, it was necessary to carry out the peal of *Candida albicans*, supplied in a tube, in the petri dish containing the sabouraud dextrose agar for colony formation and increase of the *Candida albicans* population. To perform the peal, a platinum loop was used to select *Candida albicans* and seeded on the plate using the technique of seeding of multiple streaks. The petri dish was incubated at 37 ° C and 36 hours was allowed for growth of *Candida albicans*.

To perform the inoculation of the sample, the Mc Farland scale was used. This is a nephelometric scale used with a turbidity pattern, most often used in microbiology laboratories, to determine the intensity of multiplication in culture media. This multiplication is manifested in the media by an increase of particles (fungi) that oppose the free passage of light, causing turbidity or opacity in the medium. Thus, the higher the number of fungi, the greater the opacity of the culture medium.[16]

After growth of *Candida albicans* on the petri dish using the Mc Farland scale, a colony of *Candida albicans* was selected with a platinum loop and diluted to about 10 ml of saline solution. Using a *swab*, the *Candida albicans* sample, diluted in saline solution, was seeded in a new petri dish containing the Sabouraund Dextrose Agar. The technique used was sowing by sweeping. After this, the seeded *Candida* was incubated in the oven at 37 ° C for two hours to perform the drying of the *Candida albicans* diluent. After drying the *Candida albicans*, the petri dish was removed from the oven. The yogurt was then sowed by the same technique on the petri dish containing *Candida albicans*. Finally, the compound was incubated in the oven at 37 ° C for 48 hours.

Results

Figure 1 (a, b and c) shows respectively the analysis results of: petri dishes containing Candida albicans and yoghurt; Petri dishes containing only Candida albicans (control sample); and comparing plaques containing Candida albicans alone with the plates where Candida albicans and yogurt were seeded.

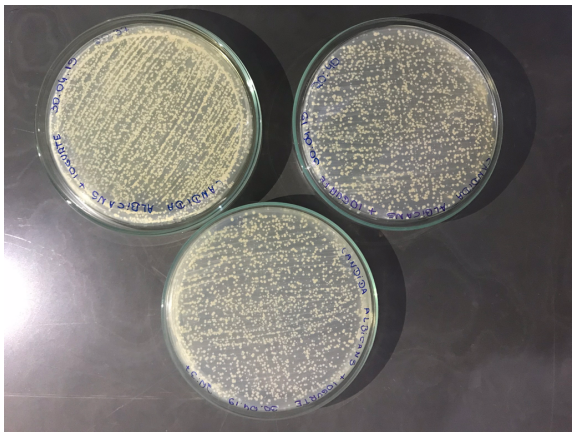

Figure 1a: Petri dishes containing Candida albicans and yoghurt.

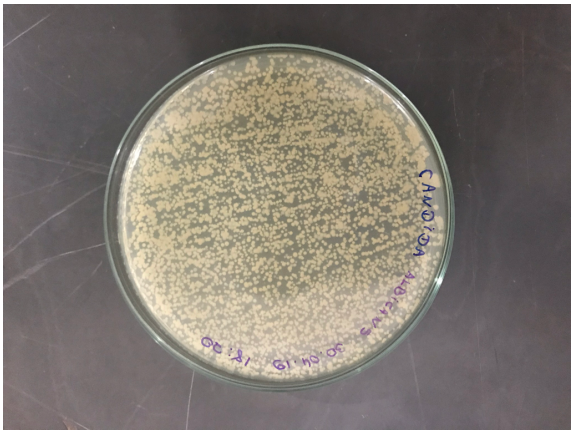

Figure 1b: Petri dishes containing only Candida albicans (control sample)

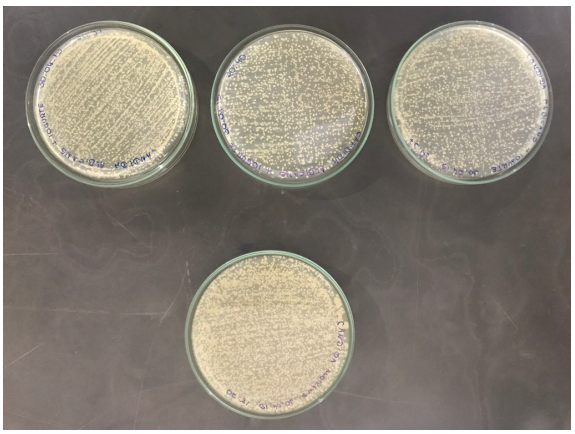

Figure 1c: Comparison of plaques containing Candida albicans alone with plates containing Candida albicans and yogurt.

After the 48h the greenhouse, compared to the control sample (Candida albicans seeded only) (Figure 1b), there was inhibition of growth of Candida albicans (Figure 1c), reducing the amount of colonies of the plaques where the yogurt was sown (Figure 1a).

Discussion

Some *in vitro* experiments have shown that some strains of *lactobacillus* may inhibit the growth of *Candida albicans* but the results of such experiments do not necessarily apply to humans because the physiological and pathophysiological mechanisms of *Lactobacillus* and *Candida albicans* in humans are very complex and can not be accurately imitated in the laboratory.[17]

Currently, there are several studies that demonstrate antifungal activity in vivo, however these results are not necessarily applied to the research object of this in vitro article. The daily intake of yogurt containing *Lactobacillus acidophilus* reduced both colonization and *Candida* infection, but the mechanism of action of *Lactobacillus* may be multifactorial.[18] Another study concluded that the consumption of yogurt associated with bee honey in patients with vulvovaginal candidiasis during pregnancy led to a relatively high level of clinical cure. This cure was even greater than that produced with the use of antifungal therapy, and the yogurt associated with honey may be used as a complementary or alternative therapy when antifungal agents are ineffective or contraindicated.[19]

In addition, some studies have shown the effectiveness of probiotics in vitro on some pathogens. In a study carried out with some probiotics, it was verified that most of the probiotics isolated in the experiments demonstrated antifungal effect against *Candida albicans*, in vitro, isolated from the oral cavity, gastrointestinal tract and genitourinary tract of humans.[20]

However, in the studies found, only one of the *Lactobacillus* investigated in this article was evaluated, namely: *Streptococcus thermophilus*. In this study, carried out in the laboratory of a private college in the state of Goiás / Brazil, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* present in the yogurt, it was identified that the *Lactobacillus* presented antifungal effect, in vitro, against *Candida albicans*.

According to ANVISA (National Sanitary Surveillance Agency), the microorganisms *Streptococcus salivarius* (subspecies *thermophillus*) and *Lactobacillus delbrueckii* (subspecies *bulgaricus*) were removed from the list of probiotics recognized by the organ. This was because, in addition to being necessary species for the production of yogurt, according to ANVISA, they do not have scientifically proven probiotic effects.[13]

Conclusion

Lactobacillus present in yogurt were effective in antifungal activity against *Candida albicans* in vitro. Few studies have been carried out in vitro, and new experiments have to be carried out to isolate *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from yogurt in order to assure the efficacy of the antifungal activity of each *Lactobacillus*.

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