



Evaluation of the physiological effect of *Saccharomyces boulardii* a candidate for the manufacture of probiotics isolated from *Garcinia mangostana* fruit pericarps

Odette N. Kabena^{2,3}, Julia N. Diakese², Jean-Jacques D. Amogu^{2,3*},
Ruth M. Katunda², Lionel S. Asambo^{1,2}, Gracias I. Mbokolo^{2,3},
Francis K. Tshiswaka² and Didier L. Dianzuangani²

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ABSTRACT

This study aimed to isolate the *Saccharomyces boulardii* strain from *Garcinia mangostana* fruit from Kinshasa; to determine its thermoresistance; and finally, to determine its antagonistic effect towards *Lactobacillus vaginalis* and *Staphylococcus aureus* to determine its suitability for use as a probiotic. The results of this study show that *Saccharomyces boulardii* isolated from *Garcinia mangostana* fruit from Kinshasa is tolerant at 44°C and resistant only at 60 and 75°C. *Saccharomyces boulardii* isolated from *Garcinia mangostana* fruit in Kinshasa has no antagonistic effect on *Lactobacillus vaginalis* and *Staphylococcus aureus*, so it lives in perfect synergy. *Saccharomyces boulardii* isolated from the fruit of *Garcinia mangostana* in Kinshasa could be a good candidate that may well contribute to the restoration of vaginal microbiota than other marketed strains. We therefore suggest that further studies on other probiotic selection criteria be carried out on the isolated strain, with a view to the scientific validation of our results.

Keywords: Microbial strain, Vaginal flora, *Garcinia mangostana*, Probiotic

¹Centers of Excellence for Nuclear, Radiological, Biological and Chemical (CoE-NRBC), Ministry of Higher Education, University, Scientific Research, and Innovation, Kinshasa, DR Congo

²Laboratory of Microbiology Applied to Biological and Natural Resources (LaMARBN), Life Sciences, Faculty of Science and Technology, University of Kinshasa, Kinshasa, DR Congo

³National Committee for Protection against Ionizing Radiation (CNPRI), Ministry of Higher Education, University, Scientific Research and Innovation, Kinshasa, DR Congo

*Corresponding author's email: jj.amogu@unikin.ac.cd (Jean-Jacques D. Amogu)

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Introduction

In cases of vaginal flora imbalance, conventional medicine tends to favor treatment with anti-infective (such as metronidazole, clindamycin, secnidazole, etc.). However, this seems insufficient, as in the event of infection, these are gradually replaced by pathogenic microorganisms, thus promoting recurrence (Lepargneur and Rousseau, 2008). The disappointing results of antibiotic treatments for recurrent vaginal conditions, as well as the pathophysiology of this infection, are leading to the increasing use of products that correct the vaginal flora,

known as probiotics (Bohbot and Lepargneur, 2012). The role of probiotics is generally to compensate for or prevent the harmful effects of dysbiosis or to temporarily mix with the microbiota to strengthen its natural functions (Rofes, 2014). Probiotic strains are introduced into the diet in the form of fermented dairy products or dietary supplements (in non-fermented products) (Bechachha *et al.*, 2020). The beneficial effects of probiotics on host health are, in theory, numerous, but scientific evidence confirming these claims requires further



investigation (Villegier, 2014). Numerous studies provide information on the use of yeast as probiotics, which are strains of *Saccharomyces cerevisiae*, and in particular a specific strain called *Saccharomyces boulardii* (Billoo *et al.*, 2006). Bohbot and Zhioua (2021) report that *Saccharomyces boulardii* is a thermotolerant yeast that grows optimally at 37°C and tolerates acids; it is a non-pathogenic microorganism initially isolated by French scientist Henri Boulard from lychee and mangosteen fruits (Hossain *et al.*, 2020). The fruit of *Garcinia mangostana* has been an integral part of Chinese medicine and Ayurveda since ancient times. The leaves and bark of the mangosteen are used in some African countries for oral care, as chewing sticks and as an astringent (Bakomba, 2020), and other studies have been conducted to isolate *Saccharomyces boulardii* from mangosteen fruit and evaluate its probiotic properties (Laffargue, 2015; Lorot, 2016). Some strains of *S. boulardii* are capable of producing high concentrations of acetic acid, which has an inhibitory effect on *E. coli* (Ducluzeau, 2002). This study aims to verify the protective effect of the microbial strain on the vaginal flora with a view to formulating probiotics. The overall objective of this work is to determine the physiological characteristics of *Saccharomyces boulardii* isolated from the fruit of *Garcinia mangostana* with a view to selecting it as a probiotic.

Materials and Methods

Plant material

The plant material used in our study consists of the epicarp of *Garcinia mangostana* fruit harvested in August 2022 from the 'Mona Paradis' concession near the N'djili Brasserie site in the Manzanza district of Kikimi-Mikondo in the commune of N'djili in the city province of Kinshasa. The figure below shows an image of *Garcinia mangostana* fruit.



Fig. 1. *Garcinia mangostana* fruit.

Microbial strains

Strains of *Lactobacillus vaginalis* and *Staphylococcus aureus* were provided by the Bacteriology Laboratory of the University Clinics of Kinshasa (CUK) and stored in a refrigerator at -6°C at the Laboratory of Microbiology Applied to Biological and Natural Resources of the Faculty of Sciences of the University of Kinshasa. The *Saccharomyces boulardii* strain was isolated from the pericarp of *Garcinia mangostana*, and ultra-yeast was used as a control strain.

Isolation

Seeding on agar medium

We soaked our *Garcinia mangostana* pericarp in physiological water for 48 hours and then seeded it in Sabouraud dextrose agar at a temperature of 29°C.

Purification

After isolation, the yeast isolates were purified using the SDA medium transfer technique. The pure cultures are stored on the same medium at a very low temperature (-6°C).

Identification

Macroscopic identification

Macroscopy consisted of observing the morphology of our isolate, including the size, shape, and appearance of colonies.

Microscopic identification

The examination was performed on fresh smears (40x magnification) prepared from fresh cultures in SDA broth.

Catalase test

The test consists of placing our isolates in contact with hydrogen peroxide (H₂O₂), then observing whether there is degradation involving the presence of catalase, which produces foam, in which case the test is positive (+); otherwise, it is negative (-) (Hidalgo-Cantabrana *et al.*, 2017).

Heat resistance test

We divided the inoculum into tubes containing Sabouraud dextrose agar poured at an angle, then incubated at different temperatures: 15, 30, 44, 60, and 75°C.

Investigation of the antagonistic effect of *Saccharomyces boulardii* against *Staphylococcus aureus* and *Lactobacillus vaginalis*

To evaluate the effect of *Saccharomyces boulardii* on *Staphylococcus aureus* and *Lactobacillus vaginalis*, we used a modified version of the method described by Marion (2018). It consists of placing discs of blotting paper impregnated with *Lactobacillus vaginalis* and *Staphylococcus aureus* inocula on the surface of a Muller Hutton medium initially seeded with a suspension of *Saccharomyces boulardii*. After incubation, the appearance or absence of a clear circular zone free of colonies around the deposited discs indicates whether or not the growth of *Saccharomyces boulardii* is inhibited in the presence of *Lactobacillus vaginalis* and *Staphylococcus aureus* (Rousseau, 2004). The presence of an inhibition zone indicates that one of the two strains in culture inhibits the growth of the other, meaning that there is antagonism between these microbial species. Whereas, if there is no inhibition zone or if the growth of the two strains in culture is mutual, there is no antagonistic effect, which is referred to as a synergistic effect. The results were read by observing the inhibition zones around the disc and measuring the diameters of these inhibition zones using a ruler.

Results and Discussion

Purification

The figure below shows an image of purified *Saccharomyces bulardii*, isolated from the pericarp of *Garcina mangoustana*.



Fig. 2. *Saccharomyces bulardii*

Macroscopic appearance

Figure 3 shows an image of purified *Saccharomyces boulardii* isolated from the pericarp of *Garcina mangoustana*



Fig. 3. *Saccharomyces boulardii*

Macroscopic examination on Sabouraud Dextrose Agar solid medium shows circular, convex, whitish colonies approximately 1 mm to 2 mm in diameter.

Microscopic appearance

Figure 4 shows the microscopic appearance of *S. boulardii*.

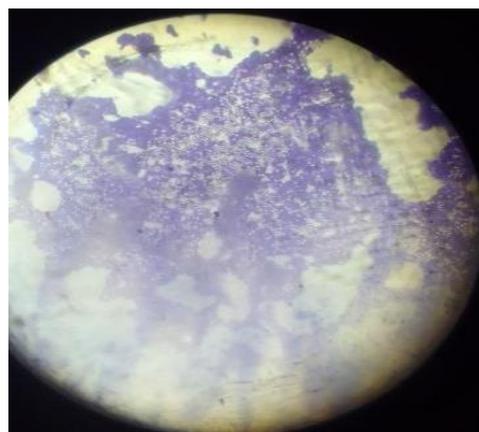


Fig. 4. Microscopic observation of the *S. boulardii* strain

Observation of *S. boulardii* shows the presence of ovoid cells with budding, indicating that they are yeast cells and that they are in a growth phase.

Catalase test

The images below show the appearance of *Staphylococci* after the catalase test.

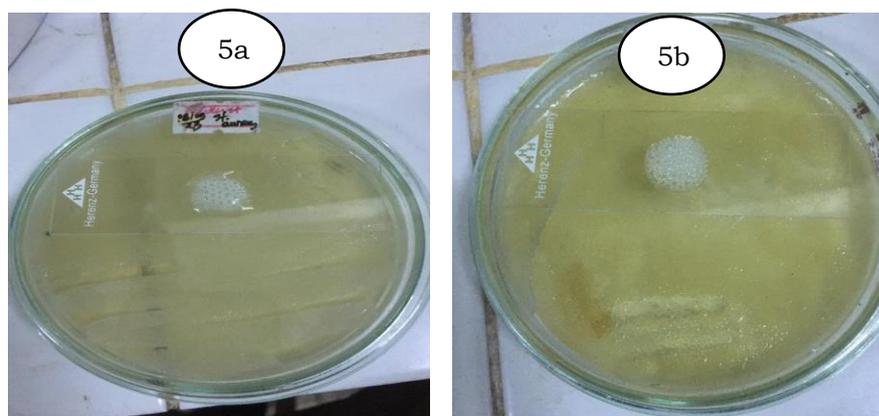


Fig. 5. LAB catalase positive and: *Staphylococcus* catalase positive.

The catalase test result was positive for all strains (*Lactobacilli* and *Staphylococcus aureus*) (gas release). *Lactobacilli* are considered catalase negative or occasionally produce very small amounts of catalase. During this work, we encountered *Lactobacillus* strains that possess catalase activity (Fig. 6).

Heat resistance of *Saccharomyces boulardii* isolated from *Garcinia mangustana*

Table 1 below shows the results of *Saccharomyces boulardii* growth at different temperatures.

Table 1. Heat resistance of *Saccharomyces boulardii*.

Strains of <i>S. boulardii</i>	Incubation temperatures				
	15°C	30°C	44°C	60°C	75°C
	+	+	+	-	-

Legend:

+: Presence of *S. boulardii* growth

-: Absence of *S. boulardii* growth

This table shows that our strain is thermotolerant up to a temperature of 44°C and cannot withstand temperatures of 60°C and above. Although *S. boulardii* is isolated from a plant, this yeast can live and withstand human body temperature (Staali, 2017). Indeed, a probiotic must be able to withstand unfavorable conditions. The work of Laffargue (2015) shows that at a temperature of 52°C, *S. boulardii* remains 65% resistant, while *Saccharomyces cerevisiae* loses up to 45% of its viability. According to our study, the heat resistance of

Saccharomyces boulardii reveals that our strain meets one of the technological criteria, which is stability.

Antagonistic effect

Analyses evaluating the antagonistic effects of lactobacilli against *S. boulardii*, ultra-yeast®, and other germs likely to be found in the vagina yielded the following results.

The images below show the results of the antagonistic effect.

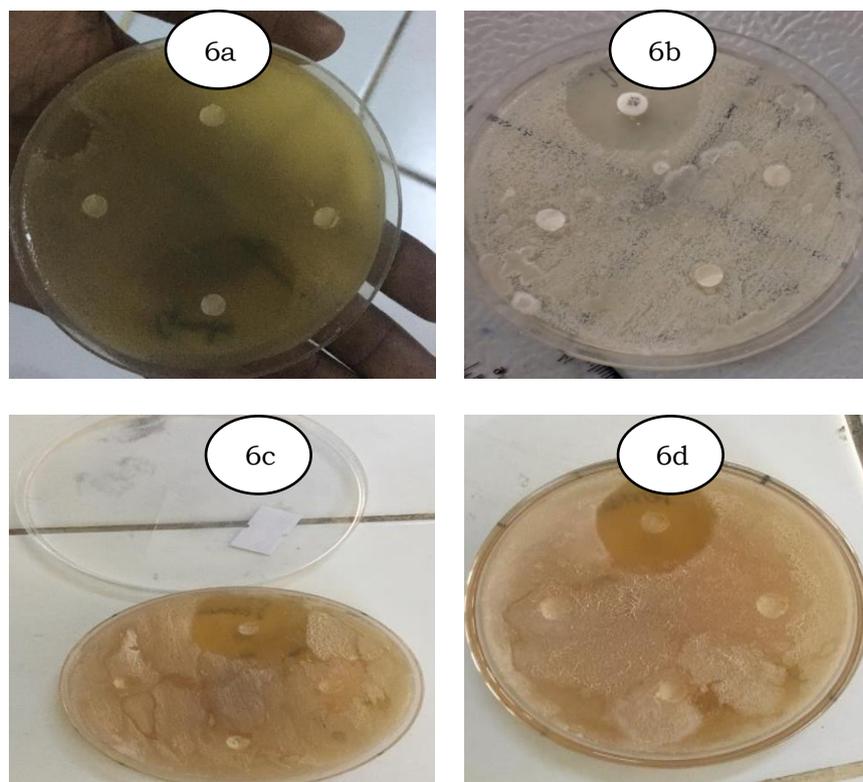


Fig. 6. Antagonistic effects.

These images show that after 48 hours of incubation, no inhibition zones were observed in the Petri dishes except for the control dishes. This observation allows us to conclude that the *Saccharomyces boulardii* strain, isolated from the fruit of *Garcinia mangostana* in Kinshasa, has no antagonistic effect on vaginal Lactobacilli.

In other words, we can say that *Saccharomyces boulardii* can coexist with *Lactobacillus vaginalis* and *Staphylococcus aureus*, thus exhibiting a synergistic effect, as reported in previous studies, including Rofes (2014) showing the symbiotic effect between Lactobacillus in the digestive flora and *Saccharomyces boulardii*.

Conclusion

This study aimed to isolate the *Saccharomyces boulardii* strain from *Garcinia mangostana* fruit from Kinshasa; to determine its heat resistance; and finally, to determine its antagonistic effect on *Lactobacillus vaginalis* and *Staphylococcus aureus* in order to determine its potential for use as a probiotic.

This study shows that:

- ✓ *Saccharomyces boulardii* isolated from *Garcinia mangostana* fruit in Kinshasa is tolerant to temperatures of 44°C and is resistant to temperatures of 60 and 75°C;

- ✓ *Saccharomyces boulardii* isolated from *Garcinia mangostana* fruit in Kinshasa has no antagonistic effect on *Lactobacillus vaginalis* and *Staphylococcus aureus*, and therefore lives in perfect synergy with them.
- ✓ *Saccharomyces boulardii* isolated from *Garcinia mangostana* fruit in Kinshasa could be a good candidate that may contribute to the restoration of the vaginal microbiota as well as other commercially available strains.
- ✓ We therefore suggest that further studies on other probiotic selection criteria be conducted on the isolated strain in order to validate our results.

Authors' contributions

All authors have read and approved the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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