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In vitro analysis of postbiotic antimicrobial activity against *Candida* Species in a minimal synthetic model simulating the gut mycobiota in obesity

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Gut fungal imbalances, particularly increased *Candida* spp., are linked to obesity. This study explored the potential of *Lactiplantibacillus plantarum* cell-free extracts (postbiotics) to modulate the growth of *Candida albicans* and *Candida kefyr*, key members of the gut mycobiota. A minimal synthetic gut model was employed to evaluate the effects of *Lactiplantibacillus plantarum* postbiotics on fungal growth in mono- and mixed cultures. Microreactors were employed for culturing, fungal growth was quantified using CFU counting, and regression analysis was used to evaluate the effects of postbiotics on fungal growth. Postbiotics at a concentration of 12.5% significantly reduced the growth of both *Candida* species. At 24 h, both *C. albicans* and *C. kefyr* in monocultures exhibited a decrease in growth of 0.11 log CFU/mL. In contrast, mixed cultures showed a more pronounced antifungal effect, with *C. albicans* and *C. kefyr* reductions of 0.62 log CFU/mL and 0.64 log CFU/mL, respectively. Regression analysis using the Gompertz model supported the antifungal activity of postbiotics and revealed species-specific differences in growth parameters. These findings suggest that *L. plantarum* postbiotics have the potential to modulate the gut mycobiota by reducing *Candida* growth, potentially offering a therapeutic approach for combating fungal overgrowth associated with obesity.

Keywords Postbiotics, Probiotics, Minimal synthetic microbiomes, Antimicrobials, Intestinal mycobiota, *Candida*

Abbreviations

SCFA	Short-chain fatty acids
SynComs	Synthetic communities
CFU	Colony forming units
OD	Optical density
YPD	Yeast extract-peptone-dextrose
MRS	De Man, Rogosa, and Sharpe
MEC	Minimum effective concentration

The gut microbiota is a complex ecosystem of microorganisms residing in the human gastrointestinal tract. This ecosystem significantly impacts the physiological health and overall wellness of the host. While bacteria have long dominated microbiome research due to their abundance and well-established roles in digestion, metabolism, immunity, and brain function[1], fungi are emerging as crucial players in this delicate ecosystem, with potential

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implications for health and disease. The gut mycobiota, encompassing diverse fungal species like *Candida* and *Saccharomyces*, has garnered increasing attention for its impact on various physiological processes[2]. Within this complex community, microbial interactions shape the growth dynamics and functionality of the gut ecosystem. These interactions are influenced by various factors like diet, age, genetics, and disease states[3]. *Lactiplantibacillus plantarum*, a prominent bacterial resident, plays a significant role in shaping the intestinal community by producing short-chain fatty acids (SCFAs) like acetic, propionic, and butyric acids[4]. In a recent study assessing the fungal species within the intestinal mycobiota of subjects with obesity, a higher abundance of *Candida albicans* and *Candida kefyr* was detected among individuals with obesity compared to those with a healthy weight; additionally, these fungal species exhibited a positive correlation with weight gain parameters, such as body mass index and percentage of fat mass, indicating an association with increased adiposity[2]. Other studies have correlated the elevated abundance of *Candida* spp. with intestinal dysbiosis[5,6]. The presence of postbiotics, bioactive molecules secreted by probiotic bacteria, frequently governs microbial interactions within the human gut microbiota. It has also been reported that these postbiotics have antimicrobial effects against pathogenic species in the human intestinal microbiota[7]. SCFAs, amongst these postbiotics, play a critical role in mediating communication between the host and the gut microbiota, influencing the gut ecosystem by driving the selective enrichment of specific bacterial populations or the decline in the growth of other gut microbes[1]. Consequently, studying the interplay between probiotic-mediated metabolite exchange, like SCFAs, and microbial interactions, including those involving intestinal yeasts, is crucial for maintaining a stable and functional gut ecosystem[1,8].

In vitro models are valuable for studying microbial growth dynamics within the human gut microbiota. These models offer several advantages, including rapid evaluation of diverse substrates, experimental reproducibility, reduced costs, and ethical considerations[9]. Recently, synthetic communities (SynComs) have been designed to mimic and model the specific compositions and responses of a portion of the gut microbiome[10]; these SynComs are laboratory-designed communities that facilitate controlled studies of specific species and their influence on the ecosystem function[11]. SynComs encompass a varying number and proportions of species, with a minimum of two required for studying growth dynamics[10,12]. Integrating these models with mathematical tools like regression analysis deepens our understanding of these dynamics in a quantitative manner, particularly those involving antimicrobial effects[13,14]. This combined approach provides a comprehensive picture of the gut's complex microorganism-spanning processes. This knowledge is crucial to harnessing the metabolic potential of the gut microbiota and mycobiota and identifying key taxa. It empowers us to modulate gut microbiota composition and function across diverse scenarios[15].

The widespread use of antibiotics and other antimicrobial agents can significantly disrupt the balance of the human gut mycobiota, leading to dysbiosis and associated health consequences. Understanding the antimicrobial properties of microbial metabolites, including postbiotics, holds immense potential for developing alternative strategies to combat pathogenic infections, modulate the gut microbiota and mycobiota, and promote overall health[16]. This study aims to investigate the potential antimicrobial effects of *L. plantarum* postbiotics within a minimal synthetic model of the human gut mycobiota, providing insights into their potential role in maintaining a healthy microbial balance and fostering gut health.

Materials and methods

Microorganisms' selection and culture conditions

Candida albicans ATCC 10231 and *Candida kefyr* ATCC 2512 were used as fungal microorganisms to represent a SynCom mimicking the intestinal mycobiota associated with obesity[2]. *Lactiplantibacillus plantarum* Lp-115 was the probiotic bacterium used for postbiotic extraction. *C. albicans* and *C. kefyr* were cultured in YPD medium (Yeast extract-peptone-dextrose), while *L. plantarum* was cultured in Difco™ MRS medium (De Man, Rogosa, and Sharpe). SynCom cultures were incubated under aerobic conditions at 37 °C with continuous shaking at 100 rpm.

Growth kinetics of *Candida* spp.

A flask containing 100 mL of fresh YPD medium was inoculated with 2 mL of a 16-h culture from each yeast, reaching an initial optical density of 0.2 at 595 nm. Subsequently, each culture was aseptically distributed into 63 microbioreactors (2 mL capacity Eppendorf-type plastic vials), each receiving 1 mL of the culture. These 63 microbioreactors served as data points for the study of the 24-h growth kinetics. The microbioreactor vials were then incubated at 37 °C and 100 rpm. The growth kinetics of the microbial cultures were evaluated by measuring the microbial concentration in colony-forming units per milliliter (CFU/mL) and the optical density of the samples. Samples were collected every hour for the first 16 h, followed by collections every 2 h until the experiment ended at 24 h. Each vial containing spent medium was centrifuged at 10,000 rpm for 5 min at 4 °C (1580R, Gyrozen). The sediment was resuspended in 1 mL of sterile peptone water (BD) to measure the optical density at 595 nm. After measuring the optical density, plate culturing was performed using the drop plate method, as described by Naghili et al. Serial dilutions were performed in peptone water using the resuspended sample, with each sample being subjected to 1:10 serial dilutions. Subsequently, 10 µL of the final dilution of each sample was aseptically spread on Petri dishes previously prepared with YPD agar for microbial growth. The Petri dishes were incubated at 37 °C for 24 h[17].

L. plantarum culture and extraction of postbiotics

L. plantarum postbiotics were obtained following the methodology described by García-Gamboa et al. (2022)[18]. *L. plantarum* was incubated in MRS broth at 37 °C and 100 rpm for 16 h. After incubation, the culture medium was centrifuged at 2800×*g* for 10 min at 4 °C to isolate the postbiotics. The supernatant was filtered through a sterile 0.45 µm pore size filter (Corning®, NY, USA) and stored at 4 °C.

Determining the dose and impact of postbiotics on *Candida* spp. growth

The minimum effective concentration (MEC) of *L. plantarum* postbiotics against *Candida* spp. was determined to select the appropriate dose for the subsequent yeast growth kinetics assay in mono and mixed cultures. *C. albicans* and *C. kefyr* were cultivated in YPD broth at 37 °C for 24 h. Cultures were then standardized to an optical density of 0.2 at 595 nm, corresponding to 1×10^5 CFU/mL. Two milliliters of each culture were added to separate flasks containing 50 mL of fresh YPD broth. Then, 1 mL aliquots were transferred to microbioreactors. Postbiotics were added to the *Candida* cultures (*C. albicans* and *C. kefyr* separately) to achieve concentrations of 12.5%, 25%, and 50%. A separate culture of each *Candida* species without postbiotics was used as a control. Cultures were incubated in triplicate at 37 °C and 100 rpm for 8 h. Growth inhibition of *Candida* spp. was monitored by measuring the optical density at 595 nm every hour[18].

Evaluation of postbiotic antimicrobial effects on *Candida* spp. monocultures and mixed cultures

This study investigated the impact of postbiotics on the growth of *Candida albicans* and *Candida kefyr*, both in mono and mixed cultures. A fixed concentration of 12.5% *L. plantarum* postbiotics was employed for monoculture and mixed culture experiments. For monoculture experiments, growth kinetics was assessed following the methodology described in the 'Growth kinetics of *Candida* spp.' section, adding 12.5% postbiotics to the growth medium for the respective *Candida* species. For mixed culture experiments, a new flask containing 100 mL fresh YPD medium was inoculated with 2 mL (OD 0.2 at 595 nm) of each 16-h culture from *C. albicans* and *C. kefyr*. The inoculated culture was aseptically distributed into 63 microbioreactors that represent the triplicates of the 24-h cultures. Microbioreactors from monoculture and mixed cultures were incubated at 37 °C and 100 rpm. Samples were collected hourly until hour 16, then every 2 h thereafter until the end of the experiment at 24 h. Microbial concentration was determined by CFU/mL using the drop plate method. Samples from monocultures were plated on YPD agar and samples obtained from mixed cultures were plated on BD ChromaAgar, a selective medium that differentiates *Candida* species by color. Petri dishes were incubated at 37 °C for 24 h. Additionally, the pH of the postbiotics and *Candida* cultures (mono and mixed) was measured using a pH meter HI 2210 (Hanna Instruments).

Modeling the growth kinetics of *Candida* spp.

To assess the antimicrobial effect of postbiotics, we analyzed the experimental data comprising the mean growth [CFU/mL] of *Candida* spp. across the eight distinct trials. The regression analysis of the data was subjected to fitting with a sigmoidal growth model, the four-parameter Gompertz model represented by Eq. (1). Gompertz model has proved an appropriate response to represent growth in batch culture processes[19], it was implemented for numerical calculation using the Curve Fitting App available in MATLAB 2023b (Math-Works):

$$y(t) = d + (a - d) \cdot e^{-e^{-b(t-c)}} \tag{1}$$

This model, renowned for its utility in microbial growth analysis[20], offers a comprehensive framework: a signifies the maximum fungal concentration, b correlates with the maximum growth rate, c denotes the termination of the lag phase, and d represents the initial concentration. We performed the regression analysis in each dataset, allowing for a comparative assessment of the set of parameters and values and the coefficients of determination R-squared. To accurately model the growth kinetics of each culture, data points for regression analysis were limited to the stationary phase, representing the sigmoidal growth pattern, determining the end of the stationary phase by calculating the second derivative to identify the inflection point of the concentration rate of change. Additionally, we derive the confidence interval (95%) for the models based on the identified parameters.

Statistical analysis

All assays were performed in triplicate, with analytical data expressed as mean ± standard deviation. The data were statistically analyzed using the non-parametric Kruskal–Wallis test using GraphPad Prism version 8.

Results

Evaluation of the impact of postbiotics on yeast growth at different doses

Postbiotics were obtained from the *L. plantarum* culture cultivated in an MRS medium until the stationary phase (16 h) was concluded to encompass the entire spectrum of metabolites secreted by the probiotic throughout its growth cycle. Afterward, the culture underwent centrifugation and filtration to ensure that the postbiotics were devoid of cells. The initial (6.50 ± 0.01) and final (3.96 ± 0.01) pH of the postbiotics are shown in Table 1. Before assessing the antimicrobial efficacy of postbiotics in monoculture and mixed cultures, we determined the minimum effective concentration (MEC) of three concentrations of *L. plantarum* postbiotics (12.5%, 25%, and 50%). All three concentrations exhibited significant reductions in the growth of both *C. albicans* and *C. kefyr* compared to the untreated control ($p < 0.05$), as determined by optical density measurements (Fig. 1). The 12.5% dose demonstrated antifungal activity against *C. albicans* and *C. kefyr*, respectively ($p < 0.001$). Absorbance data at 8 h showed a significant decrease in the growth of *C. albicans* treated with the 12.5% dose (OD = 0.74 ± 0.10) in contrast to the control (OD = 1.04 ± 0.01; $p < 0.05$). This corresponds to a reduction of 0.15 log CFU/mL (Table S1) based on a pre-established calibration curve relating absorbance to cell density. *C. kefyr* treated with the 12.5% postbiotic dose exhibited a significant decrease in absorbance (OD = 1.53 ± 0.04) compared to the control group (OD = 1.88 ± 0.04; $p < 0.05$). This corresponds to a reduction of 0.10 log CFU/mL. Consequently, the postbiotic concentration of 12.5% was chosen for subsequent experiments exploring its effects on monoculture and mixed cultures of *Candida* spp. in the minimal synthetic model of the intestinal mycobiota.

Time (h)	*Candida albicans*		*Candida kefyr*		Mixed culture		*L. plantarum* postbiotics
	Without postbiotics	Postbiotics	Without postbiotics	Postbiotics	Without postbiotics	Postbiotics	
0	6.32 ± 0.01	5.57 ± 0.02	6.30 ± 0.01	5.58 ± 0.01	6.31 ± 0.02	5.53 ± 0.01	6.50 ± 0.01
24	5.63 ± 0.03	4.90 ± 0.02	5.59 ± 0.03	4.89 ± 0.01	5.58 ± 0.02	4.86 ± 0.02	3.96 ± 0.01
pH decrease	0.69	0.67	0.71	0.69	0.73	0.67	2.54

Table 1. pH of *Candida* spp. growth in monocultures and mixed cultures and *L. plantarum* postbiotics. Values are shown as mean ± SD of three replicates.

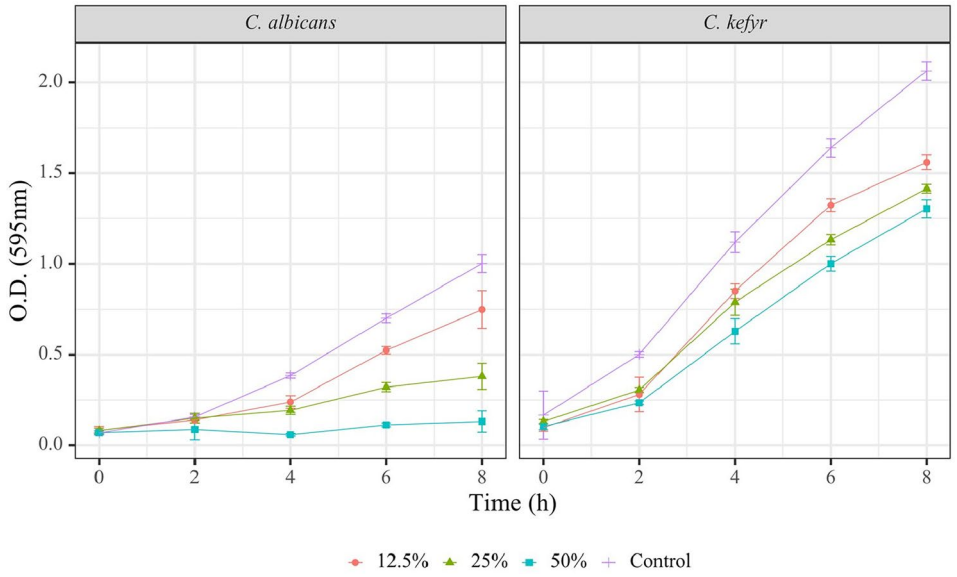

Figure 1. Antifungal activity of *L. plantarum* postbiotics by spectrophotometry in concentrations of 12.5%, 25%, and 50% against *Candida albicans* and *Candida kefyr*.

Effects of postbiotics on *Candida* spp. growth and pH in mono- and mixed cultures

The growth kinetics of *C. albicans* and *C. kefyr* in monocultures and mixed cultures, with and without postbiotics, are presented in Supplementary Material (Tables S2 and S3, respectively). Initial inoculum concentrations ranged from 5.51 to 5.69 log CFU/mL for both fungal species, with no significant differences ($p < 0.05$) between the control and postbiotic groups. In monocultures, *C. albicans* reached a maximum growth stationary phase at hour 14 (6.95 ± 0.01 log CFU/mL) without postbiotics, whereas its growth with postbiotics peaked at hour 20 (6.82 ± 0.01 log CFU/mL). *C. kefyr* achieved a maximum growth at hour 14 (7.66 ± 0.02 log CFU/mL) without postbiotics, while cultures with postbiotics exhibited a maximum growth at the same 14 h (7.54 ± 0.03 log CFU/mL). The growth patterns of *Candida* spp. in mixed cultures differed from those observed in monocultures. *C. albicans* in mixed cultures without postbiotics exhibited maximum growth at hour 11 (6.65 ± 0.01 log CFU/mL). The mixed culture with postbiotics showed a higher *C. albicans* growth (6.40 ± 0.02 log CFU/mL) at hour 13 ($p < 0.05$). Similarly, *C. kefyr* showed a maximum growth at hour 15 (7.20 ± 0.05 log CFU/mL and 7.16 ± 0.03 log CFU/mL, respectively, for cultures without and with postbiotics compared to control ($p < 0.05$). Both yeasts exhibited decreased growth in mixed cultures compared to the experiment in single cultures. *C. albicans* counts were 0.32 and 0.62 log CFU/mL lower in mixed culture after 24 h with and without postbiotics, respectively, while C. *kefyr* counts were 0.49 and 0.64 log CFU/mL lower with and without postbiotics. Both mono- and mixed cultures of *Candida* spp. exhibited a lower initial pH at zero hours (around 5.53–5.58) in the presence of postbiotics compared to cultures without postbiotics (around 6.30–6.32) (Table 1). Despite these initial differences, all cultures showed a similar decrease in pH by hour 24. Cultures without postbiotics exhibited a 0.69–0.73 pH reduction, while cultures with postbiotics showed a 0.67–0.69 pH reduction.

Mathematical modeling of the growth kinetics of *Candida* spp.

The identified parameters for each regression are presented in Table 2, along with their respective R-squared value. Based on these values, we can determine that this model was a good fit for the data as it was greater than 0.99 in most cases. The graphical representation of these regression models can be observed in Fig. 2., divided by type of culture and species. This analysis confirms what was observed with the fungal cell count, that postbiotics have an inhibitory effect in the growth of both species, regardless of the culture type, as observed when comparing the value of the maximum population (parameter *a*). In mixed cultures, both without and with postbiotics,

Parameters	*Candida albicans*							*Candida kefyr*							
	Monoculture				Mixed culture			Monoculture				Mixed culture			
	Without postbiotics		Postbiotics		Without postbiotics		Postbiotics	Without postbiotics		Postbiotics		Without postbiotics		Postbiotics	
a: Maximum population	8.86E+06	a	6.61E+06	a	4.50E+06	b	2.50E+06	4.54E+07	a	3.47E+07	a	1.60E+07	b	1.45E+07	b
b: Maximum growth rate	0.34	a	0.21	b	0.48	b	0.30	0.39	a	0.34	a	0.35	a	0.33	b
c: Lag phase duration	5.81	a	6.16	a	4.78	a	5.58	3.97	a	5.26	b	5.92	b	5.82	b
d: Initial cell concentration	4.61E+05	a	3.44E+05	a	3.57E+05	a	3.43E+05	3.01E+05	a	3.27E+05	a	3.83E+05	a	3.50E+05	a
R^2	0.9843		0.9902		0.9981		0.9785	0.9988		0.9963		0.997		0.9886	

Table 2. Gompertz model parameters estimated for *Candida* spp. growth in monocultures and mixed cultures bioreactors treated with 12.5% *L. plantarum* postbiotics. The values of parameters *a* and *d* are expressed as CFU/mL, while parameter *b* is expressed as CFU/mL/h. Parameter *c* values are expressed in hours (h). Statistically significant differences (Kruskal–Wallis test with a significance level < 0.05) are denoted by distinct letters (a, b, and c) within each treatment row for each yeast.

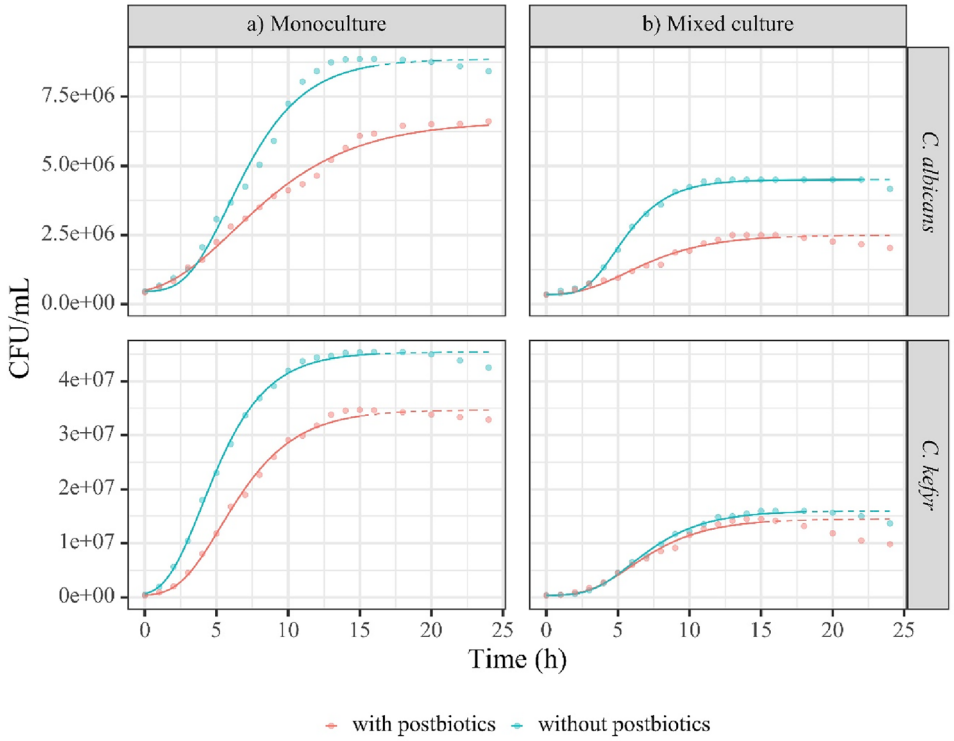

Figure 2. Four-parameter Gompertz regression of culture experimental data of *Candida* spp. with and without postbiotics (12.5%) derived from *L. plantarum*. Teal: culture without postbiotics, Red: culture with postbiotics. Columns: type of culture. Rows: *Candida* species.

significant differences were observed in the maximum population for both *C. albicans* and *C. kefyr* (4.5E + 06 and 2.5E + 06 CFU/mL, respectively, for *Candida albicans*; 1.6E + 07 and 1.45E + 07 CFU/mL, respectively, for *C. kefyr*) ($p < 0.05$). The impact on the maximum growth rate, denoted by (parameter *b*), differed within the *C. albicans* cultures. During monoculture with postbiotics, this parameter exhibited a decrease (0.21 CFU/mL/h). In contrast, in mixed culture without postbiotics, it showed an increase (0.48 CFU/mL/h), indicating a significant statistical difference compared to the control (0.34 CFU/mL/h) ($p < 0.05$). Notably, it is important to remark that the culture without postbiotics alongside *C. kefyr* was beneficial in this parameter for *C. albicans*. Conversely, *C. kefyr* in monoculture showed no significant change with or without postbiotics. However, in the mixed culture

with postbiotics, it decreased the maximum growth rate (0.33 CFU/mL/h, compared to the control's 0.39 CFU/mL/h, $p < 0.05$).

Postbiotics affected the lag phase duration (parameter *c*) of *Candida* spp. In mixed cultures, *C. albicans* experienced a shortened lag phase (reduced by 1.03 and 0.23 h in the cultures without and with postbiotics, respectively, although not statistically significant). Conversely, lag phase duration was prolonged for *C. kefyr* in both mono- and mixed cultures with postbiotics ($p < 0.05$). Specifically, the increase in lag phase for *C. kefyr* was 1.29 h in monoculture with postbiotics and 1.95 and 1.85 h in mixed cultures without and with postbiotics, respectively. Further analyzing the results from the regressions, specifically the 95% confidence interval for prediction of the parameters, for the case of *C. albicans*, it can be determined that for the monoculture, the growth kinetics can be modeled differentially up to 6.8 experimental hours. For previous time points, the models overlap (Fig. 3a); however, during mixed culture, this time came earlier at 4 h (Fig. 3b). This allows us to infer that the ecological interactions during mixed culture accelerate the antimicrobial effect of postbiotics compared to monocultures for this species. In the case of *C. kefyr*, clear differences are evident during monoculture, beginning as early as experimental hour 2.2. However, the model overlaps during the experiment duration for the mixed culture (Figures S1a and S1b). This indicates that ecological interactions play a major role in the effect that postbiotics will exert on this species.

Discussion

This study explored the potential application of *Lactiplantibacillus plantarum* postbiotics as antifungal agents against *Candida albicans* and *Candida kefyr*, key members of the human gut mycobiota. We employed a minimal gut model with these two yeasts relevant to obesity. Changes in the composition of the gut mycobiota, particularly an increase in *Candida* spp., have been linked to various pathologies and intestinal dysbiosis[21]. Recent studies have shown alterations in the gut mycobiota of obese individuals, with a rise in *C. albicans* and *C. kefyr* species potentially contributing to obesity development[2,22]. The potential pathogenic role of *Candida* spp. in obesity has also been explored, with their high prevalence and association with fungal dysbiosis being implicated[23]. The diverse *Candida* species in the intestinal microbiota possess a variety of enzymes that act as potent virulence factors, enabling them to survive and thrive. These enzymes, including phospholipase, esterase, proteinase,

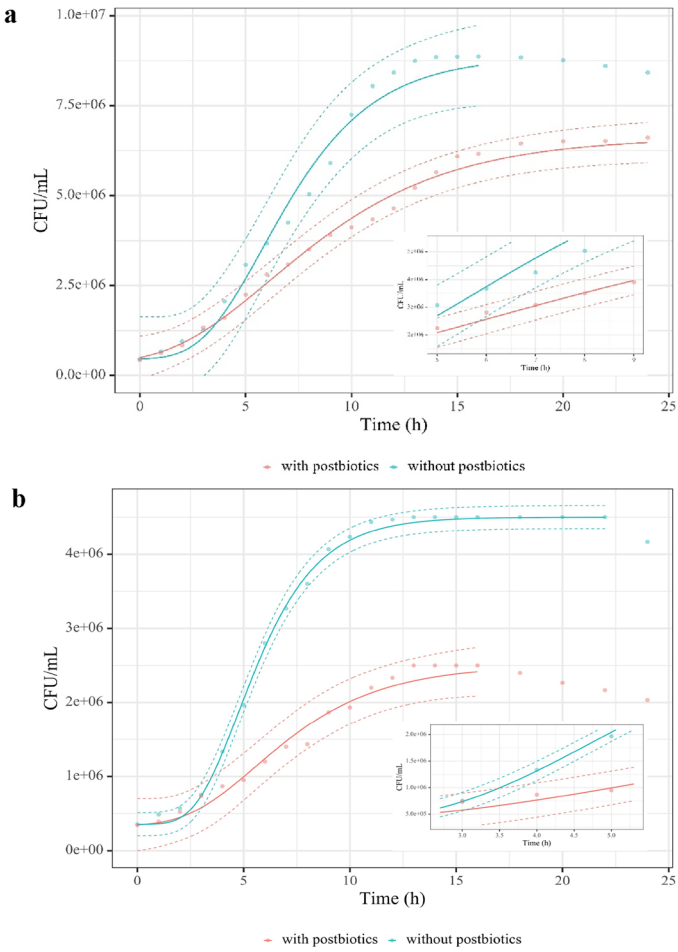

Figure 3. Analysis of 95% confidence interval of model parameters for *C. albicans* monocultures (**a**) and mixed cultures (**b**) with and without postbiotics (12.5%) derived from *L. plantarum*.

caseinolytic protease, hemolysin, and coagulase, facilitate host cell damage, nutrient acquisition, and immune system evasion[24].

Furthermore, administering specific probiotics like *Lactobacillus* and *Bifidobacterium* or their postbiotics may help restore intestinal microbiota balance and control *Candida* spp. overgrowth[25,26]. *L. plantarum*, for instance, has effectively inhibited the growth of various prokaryotic intestinal pathogens, including *Helicobacter pylori*, *Salmonella* spp., *Listeria monocytogenes*, and *Clostridium difficile*[27]. The findings of this study, where *L. plantarum* postbiotics inhibited *C. albicans* and *C. kefyr*, align with previous research indicating their ability to suppress the growth of yeast-like intestinal pathogens such as *Candida* spp[28]. The production of lactic acid, short-chain fatty acids such as acetic, propionic, and butyric acids (expected at concentrations of approximately 13.43, 1.75, 0.23, and 1.85 mM, respectively)[29], and bacteriocins like plantaricins by *L. plantarum*, has been linked to this inhibitory effect against *Candida* spp. and other intestinal pathogens[30].

Susceptibility to antifungals varies among *Candida* species. In this study, the observed difference in susceptibility between *C. albicans* and *C. kefyr* to *L. plantarum* postbiotics may be related to possible species-specific mechanisms of action and interactions between the species when co-cultured. Our findings demonstrate promising antifungal activity of postbiotics against *Candida* spp., particularly in co-culture, suggesting their potential role in modulating the intestinal fungal community. The observed decrease in growth of both *Candida* species in mixed cultures compared to monocultures could be attributed to competition for nutrients, production of specific antimicrobial compounds by the microorganisms, or other mechanisms related to the phenomenon of 'resistance to colonization'[23]. Furthermore, some *Candida* species exhibit a reduced capacity to develop biofilms when co-cultured, which may contribute to their greater susceptibility to antimicrobials[31–33], along with competition and potential antimicrobial production. It is essential to highlight these aspects, as in the intestinal microbiota, microbe-microbe and microbe-host interactions develop, meaning interactions depend not only on the growth of a single microorganism but also on interaction with the entire intestinal environment. Hence, using minimal synthetic intestinal models is crucial for evaluating the antimicrobial effect of *L. plantarum* postbiotics against gut pathogens. These models allow monitoring of microbial growth while considering the interactions of a minimal set of assembled species representing a human gut ecological niche[34].

The regression analysis revealed high R-squared values observed across most conditions, indicating a robust fit of the Gompertz model to the experimental data concerning the growth dynamics of *Candida* spp.[35]. This aligns with the previous work of Guo et al.[36], where the Gompertz model effectively described microbial growth in the gut microbiota. Postbiotics consistently demonstrated inhibitory effects on both fungal species, as reflected by lower values of the maximum population, irrespective of the culture type, thereby emphasizing the potential antifungal properties of postbiotics against *Candida* spp.[37]. Postbiotics significantly modulated microbial growth, extending the lag phase in *C. kefyr* monocultures and mixed cultures. This delayed onset of growth could be due to physiological adaptations required to cope with suboptimal conditions or the need to adapt to a new environment[38]. These lag phase adaptations are likely linked to the development of tolerance mechanisms in response to the antimicrobial stress imposed by postbiotics[39]. The observed lag phase differences between *C. kefyr* and *C. albicans* suggest differential interactions between postbiotics and *Candida* species, possibly influenced by culture conditions[40]. Postbiotics exhibited species-specific effects on maximum growth rate[35]. In *C. albicans* monoculture, they caused a decrease, while for *C. kefyr*, the decrease was observed only in mixed culture, suggesting differential response mechanisms among *Candida* species[41]. Interestingly, in mixed cultures without postbiotics, the presence of *C. kefyr* positively influenced *C. albicans* growth rate, suggesting potential interactions between these fungal species[42].

Recognizing the limitations of this study, we used a simplified in vitro minimal synthetic model with only two fungal species from the human gut microbiota. Future research should utilize more intricate synthetic models to strengthen our antifungal observations and explore a broader spectrum of inhibitory properties, such as biofilm inhibition against *Candida* species. These models should incorporate a wider diversity of gut microbiota, including relevant bacterial species, and mimic the gut's anaerobic environment. Additionally, elucidating the specific mechanisms by which postbiotics exert their activity remains a crucial area for further investigation. One approach to explore these interactions further is by incorporating models like Lotka-Volterra to analyze the specific types of ecological interspecies interactions (e.g., competition, commensalism) developing between *C. albicans* and *C. kefyr*.

Conclusion

In conclusion, this study explored the potential of *Lactiplantibacillus plantarum* postbiotics to inhibit the growth of *Candida albicans* and *Candida kefyr*, fungal members of the gut microbiota associated with obesity. Using a minimal gut model, we found that postbiotics reduced the growth of both yeasts. In monocultures, the reduction was 0.11 log CFU/mL for both *C. albicans* and *C. kefyr*. The postbiotics exhibited a stronger inhibitory effect on the yeasts in mixed cultures, reducing their growth by 0.62 log CFU/mL and 0.64 log CFU/mL for *C. albicans* and *C. kefyr* respectively. These findings suggest that postbiotics could be beneficial in modulating the gut fungal community and combating fungal overgrowth associated with obesity. The Gompertz model effectively captured the growth dynamics of *Candida* spp., with postbiotics consistently demonstrating inhibitory effects.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions
The authors confirm contribution to the paper as follows: R.G.G. and A.G.G. were responsible for conceptualization, investigation, analysis, and writing. J.G.G. and M.J.A.C. were responsible for experimental procedures and methodology. Y.P.A. was involved in conceptualization, supervision, and critical review. A.G.V. performed the mathematical analysis. All authors have read and agreed to the published version of the manuscript.

Competing interests
The authors declare no competing interests.

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