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Pro, para and post-biotic properties of lactic acid bacteria: effect on biofilm-forming pathogenic yeasts

Soutenu publiquement, le :

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Dedicace

With all my love and respect, even though words can never fully express what's in my heart and the extent of my gratitude to you, I dedicate this work and success to you all

To my parents, who have always been my support and foundation:

*My mother **ZOYBEIDI FADHILA**, who has consistently ensured my comfort, whose prayers have always accompanied me and protected me from all harm.*

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I dedicate this work to all of you, with my deepest gratitude and love. Your support, prayers, and encouragement have been the cornerstone of my success. Without you, this achievement would not have been possible. You have been my strength in times of weakness, my light in moments of darkness, and my motivation to persevere.

To my parents, your sacrifices have not gone unnoticed. The comfort you've provided, the prayers you've offered, and the unwavering belief you've had in me have been my guiding stars.

IKRAM

LISTE OF ABBREVIATION

PBS: Phosphate Buffered Sline
ATCC: American Type Culture Collection
LAB: Lacric Acid Bacteria

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GÉNÉRAL

INTRODUCTION

A class of fungi, the pathogenic yeasts, is a group of microbes that can cause an extensive range of diseases in both humans and animals. these microbes were said to have developed several strategies to invade and colonize host tissues, leading to the production of clinical symptoms. the outcome, either localized or systemic, will depend on the species of the pathogenic yeast and the immunological condition of the host. Often involving the nails, skin, and mucous membranes, the nature of these superficial infections usually causes a lot of pain and frequently appears with visible signs such as redness, itching sensations, and abnormal discharge. In severe cases, these pathological yeasts disseminate through the bloodstream to several organs, after which fatal systemic infection may ensue. They are also causative of allergic responses or their toxins dangerous to the health of a host ***(Cannon ,2022).***

Of the pathogenic yeasts, Candida albicans is considered to be of high prevalence and in addition to that well-characterized. As an opportunistic pathogen, it belongs to the usual mucosal flora of the human microbiota, not leading to disease in healthy individuals. Howbeit, it changes from a commensal to a pathogenic one, especially under weak immunity conditions, microbial dysbiosis, or shifts in local environments. Infections caused by C. albicans range from superficial candidiasis- Thrush and vaginal yeast infection-to the higher-order invasive candidiasis in various body organs ***(Berman and Krysan, 2020).***

C. albicans has several virulence factors that enable it to grow in a variety of host environments and actuate pathogenicity. Adhesins are expressed that mediate attachment of the yeast to host surfaces, secretion of hydrolytic enzymes breaking down host tissues takes place, and dimorphic suitability to switch between yeast and hyphal morphologies. It is in the hyphal morphology particularly that there is relevance to tissue invasion and biofilm formation. Furthermore, C. albicans is competent to produce a lot of toxins and immunoregulatory compounds that impede the host's defense mechanisms. The key element responsible for providing persistence to this pathogen and making it highly resistant to antifungal treatments is the ability to form biofilms on biological and abiotic surfaces. These virulence factors, coupled with the adaptability of the yeast to different environmental stresses, make C. albicans an extremely formidable pathogen capable of inducing mild to serious diseases ***(Zhou et al ., 2017).***

Recent research efforts have increased in search of new strategies to fight *Candida albicans* infections in view of the growing concerns of antifungal resistance. Researches target different strategies, which have unique biological features of the yeast and its interaction with the host

environment as a target. One of the promising avenues that is gaining attention is the use of probiotics as a potential solution (**Pérez-Sánchez et al, 2014).** Beneficial microorganisms known as probiotics show potential for the management of mechanisms preventing adhesion or production sites of yeast, antagonism of the growth of *C. albicans*, and modulation of the host immune response can be applied to probiotics for balance against *C. albicans* overgrowth (**Fijan, 2014).** Such probiotics may also counteract another key virulence factor of *Candida;* biofilm disruption. such appeal of probiotics comes from the fact that they are natural, likely to have less possibilities of adverse reactions when compared with classic antifungals, and the long-term colonization that would give long-lasting protection against *Candida* overgrowth. Although this would require further studies to establish clinical effectiveness, such possibilities certainly give a tremendous promise in the potential for probiotics to become alternative treatments or combinational therapies for Candida infections (**Salinas et Elías 2020).**

Probiotics are live microorganisms that provide a health advantage when administered in adequate amounts to a host (**Marco et al., 2017).** Beneficial bacteria and yeasts usually originate from food, the human microbiome, and environmental samples. Classical sources for probiotics are dairy products, yogurt, kefir, fermented vegetables, sauerkraut, kimchi, and specially formulated dietary supplements (**Sornplang et Piyadeatsoontorn, 2016).** Researchers have also isolated probiotic strains from the gastrointestinal tracts of healthy individuals and different ecological niches (**Walter et Ley, 2011).**

These beneficial aspects of probiotics are very diversified and constantly researched. These microorganisms help in maintaining a balanced gut microbiota, which is necessary for the well-being of an organism **(Hill et al., 2014).** Probiotics enhance gastrointestinal health by improving digestion and absorption **(Bron et al., 2011),** reducing intolerance to lactose **(De Vrese et Marteau, 2007)**, and reducing incidence and duration of diarrhea **(Marteau et al., 1990)**. They also support immune function, hence reducing the risk of specific infections and allergies **(Kumar et al., 2012).** Some studies even stipulate that they can have positive influences on mental health regarding mood regulation and cognitive function through the gut-brain axis ***(Singh et al., 2020***). Besides that, findings discovered that some strains of probiotics have the special talent of producing vitamins **(O'Mahony et al., 2005),** reducing cholesterol levels **(Karczewski et al., 2010),** and even giving better skin condition **(Cross, 2002)**. Although the exact benefits differ from strain to individual, the accumulation of evidence acts in favor of the potential for probiotics to be a very valuable tool for the promotion of general health and well-being.

Our main objective is to develop a more effective strategy for controlling *C. albicans* biofilm. In specific, we aim to investigate different biotic formulations derived from lactic acid bacteria as new anti-*candida albicans* agents in both their planktonic and sessile aspects.

CHAPTER I

MATERIAL AND METHODS

I.1. Objectives Of The Work

I.1.1. General objective

The aim of this work is to develop an effective treatment to inhibit *Candida albicans and* to explore the potential of lactic bacteria *S.thermophilus* and *L.plantarium,* not only in their live probiotic form but also through their killed form (parabiotic properties) and their cellular components (postbiotic properties), in the fight against *C. albicans* infections. Particular emphasis is placed on the inhibition of biofilm formation, a key factor in the pathogenicity and resistance to treatments of this yeast.

I.1.2. Specific objectives

- Characterization of the probiotics: This was done to evaluate the probiotic potential and survivability of certain selected lactic strains as *S.thermophilus* and *L. plantarium* under intestinal conditions. This includes thermoressitance, acidity resistance, resistance towards bile salts, antibioresistance, and antibacterial effect.

- Investigation of the anti- *C. albicans* activities*:* biotic formulation effect on growth and total biofilm formation.

I.2. Location and Period of Work

This work was carried out from February 2, 2024 until may 2 ,2024, within the microbiology and biochemistry Laboratories of the Faculty of Nature and Life sciences at Ibn Khaldon University in Tiaret.

I.3. Material

I.3.1. Biological material

I.3.1.1. Bacterial Strains

Two lactic acid bacteria were used

- *Strepthococcus thermophilus* : Isolated from yogurt by our supervisior Boubakeur badra
- *Lactobacillus plantarum* 299V: a commercial probiotic

I.3.1.2. Motivation for choosing strains

Among the broad range of probiotic microorganisms, special attention has been given to single strains of *Streptococcus* and *Lactobacillus plantarum* (**De Vrese et al., 2007).** Some species of the *Streptococcus* genus, mainly *Streptococcus thermophilus*, are commonly used in yogurt production and have been shown to exert probiotic effects such as enhancement of lactose digestion or immune modulation (**Kumar, 2012)**. On

the other hand, *Lactobacillus plantarum* is an all-versatile probiotic strain residing in a myriad of fermented foods and is currently being investigated for its potency in enhancing gut barrier function, reducing inflammation, and hence possibly alleviating symptoms of irritable bowel syndrome (**Singh, 2020).**

I.3.1.2. Pathogenic yeasts

- *C. albicans* ATCC10237
- *C. albicans* ATCC 10231

I.3.2. Equipment And Products

The equipment (apparatus, glassware) and products (growing medium and chemicals, colorants) used for this work are listed in **Table 1**

Table 1: Equipment and Products Used

Equipment	**Glassware**	**Growing Medium**	**Chemical Product**	**Colorants**
- Magnetic Stirrer -Balance -Centrifuge -Vortex Mixer -Refrigerator -Optical Microscope -uv visible - Spectrophotometer -Water Bath -pH Meter -Bunsen Burner -Incubator	-Petri Dishes -Watch Glasses -Spatulas -Wash Bottles (Pissettes) -Beakers -Pasteur Pipettes -Test Tubes -Slides -Test Tube Racks -Micropipettes	-MRS Agar and Broth -Mueller-Hinton Agar -MIT Agar -Sabouraud Agar and Broth	- PBS (Phosphate Buffered Saline) -NaOH (Sodium Hydroxide) -HCl (Hydrochloric Acid) - Methanol99% -Acetic 33% Acid -Bile Salts -Ethyl Acetate -Chloroform -Xylene -Glucose -Pepsin 5% Saline Solution Antibiotic Disks	-1%Crystal Violet -Fushsine -Gentian Violet -Lugol's Iodine

I.4. Methods

I .4.1 Experimental Approaches

The experimental approach towards conducting the above-mentioned research can, therefore, be summarized in the following

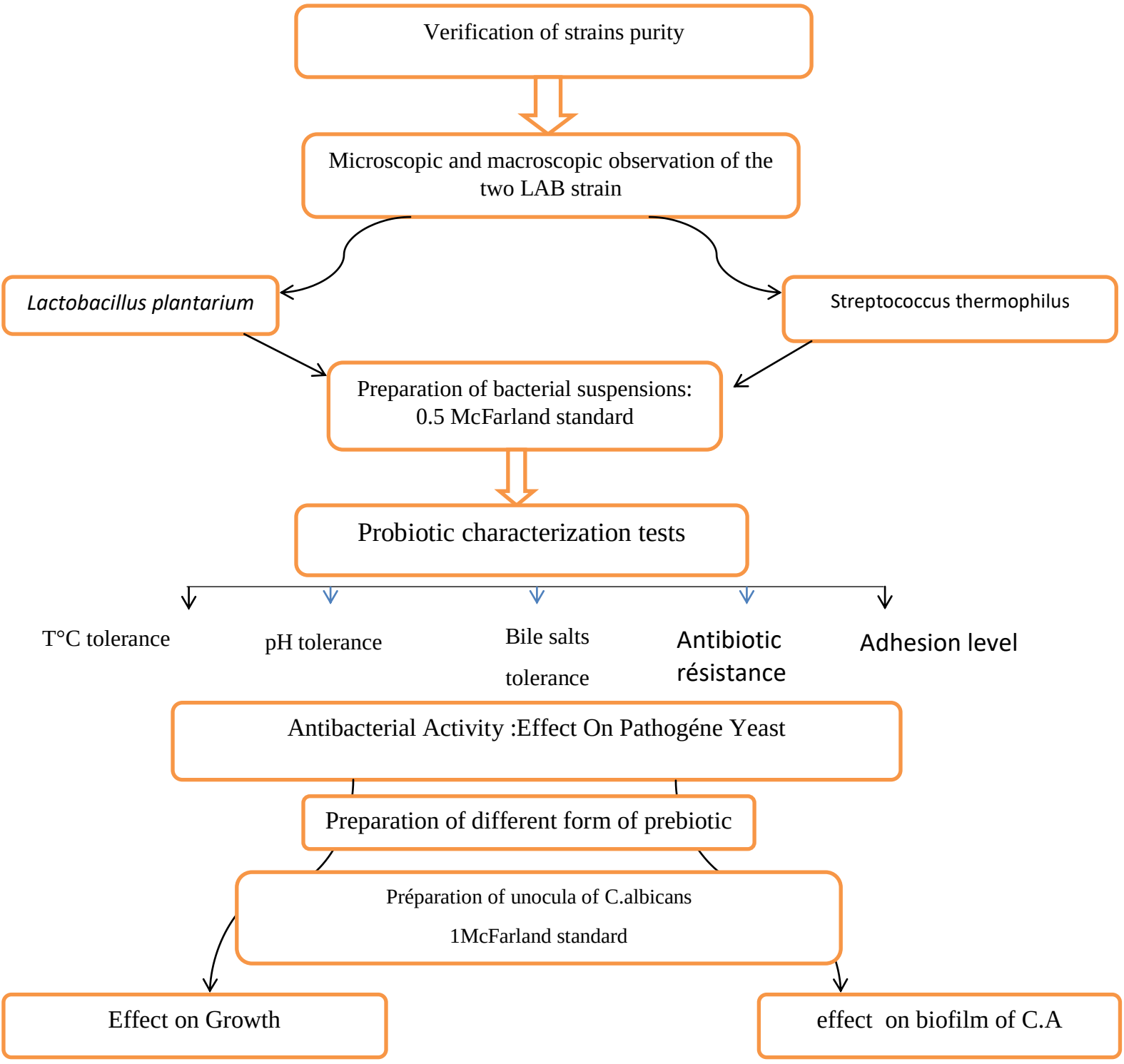


Figure 01 : Experimental protocol

1.4.1.1 Verification of strain purity and preparation of inocula

After being stored for a week, the two lactic acid bacteria *S.thermophilus* and *L. plantarium* needed to have their purity confirmed by a macroscopic and microscopic examination.

In order to prepare the inocula, young cultures (18 hours) were performed on gélose MRS. Two series of tubes were then prepared and inoculated with a young colony of *S. thermophilus* or *L. plantarium*, incubated, and preserved. The suspensions are reactivated and used to prepare the inocula according to Mac Farland's method at echelle 0.5 before each use ***(Andrew, 2008).***

I .4.2. Probiotic Properties

I 4.2.1 Tolerance to Bile Salts

The protocol for determining the bile salt resistance of the two bacterial strains, *S. thermophilus* and *L. plantarum*, followed the method described by ***Boubakeur et al.(2021).*** MRS media containing bile salt concentrations of 0.05%, 0.1%, and 0.2% were prepared and dispensed into a series of tubes, each containing 9 mL. To each series, 1 mL of inoculum with a fixed bacterial load of 10^7 CFU/mL was added. The tubes inoculated with *S. thermophilus* were then incubated for 24 hours at 42°C, while those containing *L. plantarum* were incubated at 37°C. Following the incubation period, bacterial growth and resistance to the different bile salt concentrations were evaluated by measuring the optical density at 570 nm.

I 4.2.2. Acid pH tolerance

The test follows the protocol determined by ***Boubakeur et al.(2021).*** The bacterial resistance to simulated gastric conditions for the two lactic bacteria is assessed by first preparing a fresh 18 hour bacterial culture, centrifuging it at 6000g for 20 minutes, decanting the supernatant, and washing the cell pellet three times with PBS. A simulated gastric juice solution is then prepared by adding 0.3% pepsin to 0.5% physiological saline and adjusting the pH of the solution to 2.0 and 3.0 using a pH meter

to mimic gastric pH conditions. The washed bacterial suspension is inoculated into the simulated gastric juice solutions at pH 2.0 and 3.0. The optical density of the inoculated solutions is measured at 570nm to obtain the initial bacterial count. The solutions are then incubated under the simulated gastric conditions for a desired period, typically 3 hours. After incubation, the optical density is measured again at 570nm to determine the final bacterial count.

I 4.2.3 Thermotolérance

The thermotolerance test of the two lactic bacteria *S. thermophilus* and *L. plantarum* was evaluated according to the protocol described by ***Boubakeur et al. (202*1).** A fresh 18 hour bacterial culture adjusted to a bacterial load of $10^{\wedge 7}$ CFU/ml was added to a series of tubes with MRS broth medium. The series of tubes were incubated at different temperatures; *S. thermophilus*: 42°C, 60°C and 90°C for 24h , 2h and 30 min respectively , for *L. plantarum* 37°C for 24h , 60°C for 2h and 90°C for 30 min. The optical densities were measured at 570nm.

I 4.2.4.Antibiotic Resistance

Using the disc diffusion method as outlined by ***Boubakeur and al. (2021),*** antibiotic resistance was examined; 0,1µL of inocula was added on gélose MRS. A total of six antibiotics were tested: cefeprime, gentamicin, tetracycline, colistin , chlorophenicol and metronidazole. *For L. plantarium* and *S. thermophilus,* the inhibitory zone diameters (mm) were determined following a 24 hour incubation period at 37°C and 42°C, respectively.

I.4.3. 5. Bacterial Adhesion

I.4.3.5. 1. Auto-Aggregation and Co -Aggregation

The aggregate capacity was estimated using the ***Boubakeur and al.,2021)*** and ***Khaedm and al.,2020)*** methods with little modification; the bacterial biomass from the 18 h fresh cultures was extracted using centrifugation at 5000 g for 15 min and washed three times with PBS. Subsequently, the suspension of cells was adjusted to a final charge of 10^8 CFU/mL and divided into 5 tubes, each holding 4ml. After the tubes were decanted for five hours, ODs at 578 nm were measured every hour. The following formula was used to quantify the data as a

percentage: % Autoaggregation is equal to 1-(A_t/A_0).100, where After a one-, two-, three-, four-, or five-hour decantation, the suspension OD is at (A_0), or t_0. For co-aggregation, the same method was followed. After adjusting the suspension to 10^8 CFU/mL, distribute the two lactic bacteria in the same series of tubes. The OD was measured after each hour at 570 nm, and the results were expressed as a percentage according to the same formula.

I 4.4 Biotic formulation effect on Candida albican

I 4.4.1 Preparation of different form of prebiotic

The following are the protocols to prepare probiotics, parabiotics, and postbiotics: Probiotic Preparation: Fresh culture of the two LAB strains *S.thermophilus* and *L.plantarium* grown for 18 hours. Carry out cryocentrifugation at 10,000g for 20 minutes at 4°C. and washed twice in PBS***(Zheng, X.et AL.,2018)*** .

Parabiotic Preparation : Grow a fresh culture of LAB strains . Put the culture in a water bath at 80°C with intermittent shaking every 5 minutes for 15 minutes. This allows for equitable heat distribution and the rupturing of the inactivated probiotics (***Lee, Y. K., & Salminen, S. ,2009)***

Preparation of Postbiotics: After the fresh culture has been prepared, cryocentrifugation needs to be performed at 10 000g for 20 minutes at 4°C. Recovery of supernatant after cryocentrifugation containing postbiotics ***(Aguilar-Toalá, J. E., et al.,2018)***

I 4.4.2.Effect on growth

The antibacterial activities of pro, para, and postbiotics from two LAB strains against *M3: Candida albicans* ***ATCC1023*** and *CA: Candida albicans* ***ATCC10231*** in planktonic cultures were assessed according to the methodology previously described by(***Lin et al.,2015***), with some modifications. In a microplate, 100 µl of a fresh bacterial suspension of *Streptococcus thermophilus* was inoculated into the first series of wells. Subsequently, each series was inoculated with LAB strain(synergetic effect), parabiotic, postbiotic, and probiotic solutions of *S. thermophilus*, followed by the addition of 100 µl of the *C. albicans* test strain with concentration of 10^6, 10^7. The same procedure was repeated for *Lactobacillus plantarum*

and its respective pro, para, and postbiotics. The microplate was then incubated for 24 hours at 37°C.

I 4.4.3.Effect on biofilm of *C. albicans*

In this work, biofilm formation was performed in 96-well plates by the modified method of ***Rossoni et al. (2018***). To begin, add 100 µl of a standard suspension containing cells of *Candida albicans* in a quantity of 10^7 and 10^6 cells/ml in the well. Incubate the plates at 37°C for 90 minutes. Further, wash the wells twice with PBS. Afterwards, add 50 µl culture suspension of *Lactobacillus plantarum* and *Streptococcus thermophilus*. Use 50 µl PBS or MRS medium to set up control groups. Add 100 µl MHz medium in each well, and then put the plates in the incubator for 48 hours at 37°C, refreshing the medium after 24 hours. After 48 hours of biofilm formation, the well content was aspirated and the wells washed twice with PBS. Then 200 µl of PBS was added to each well and incubated for 24 hours at 37°C. The biofilms were fixed with 100 µl of 99% methanol for 15 minutes; later on, they were washed twice with PBS. The biofilms were stained with 100 µl of the 1% crystal violet solution for 20 minutes and washed using PBS as excess stain remover. This bound crystal violet was further solubilized in 150 µL of the 33% acetic acid. Later at 540 nm, the absorbance was read against the blank using an ELISA.

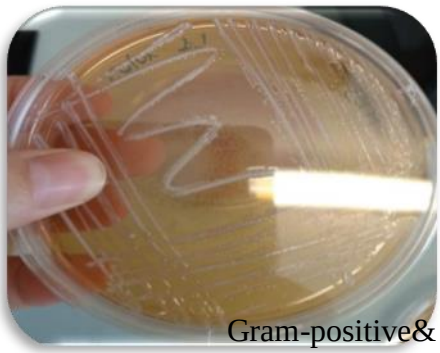
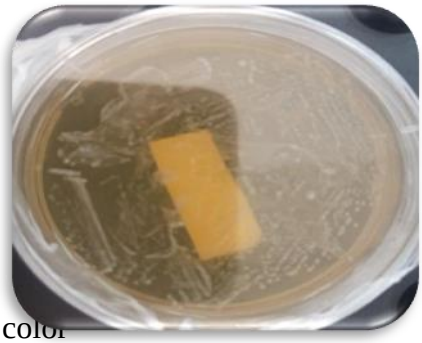
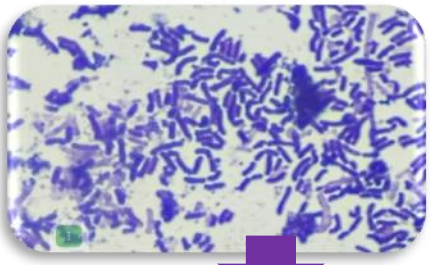
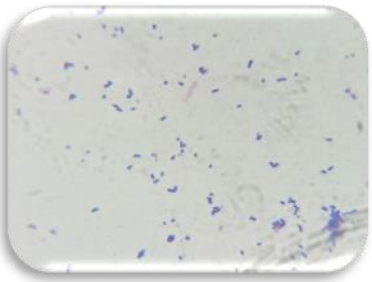
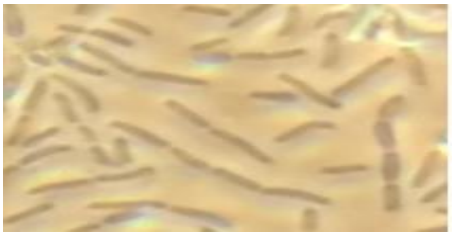
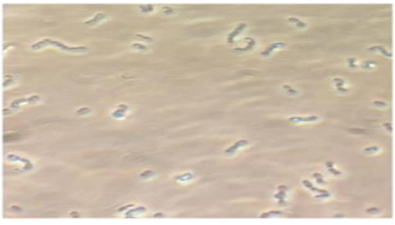
CHAPTER II

RESULTS AND DISCUSSION

Results And Discussion

II.1. Result Of Verification Of Strain Purity

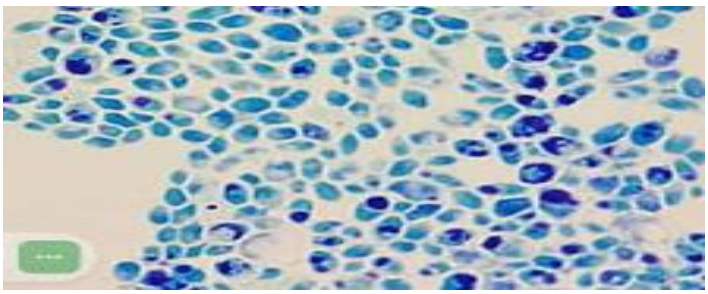
The following tables shows the macroscopical and microscopical observations of the two studied souches.

Characteristics	***L. plantarum* 299v**	***S. thermophilus***
Macroscopic Aspect (photo prise par Boubakeur)		
Microscopic Aspect « Gram staining» (photo prise par Boubakeur, 22/04/2024)	Gram-positive& purple cells color 	
Contrast microscopic treatment (photo prise par Boubakeur, 03/05/2024)		

Association Mode	Short chains of bacilli	Long chains of Cocci

Table 02: Microscopic and macroscope observation for the verification of both lactic strains

Table 03: Microscopic and macroscope observation for the verification of both used yeast

Characteristics	***C. albicans* ATCC10237**	***C. albicans* ATCC 10231**
Macroscopic Aspect (photo prise par Benmessoud, ../../2024		
Microscopic Aspect « Gram staining» (photo prise par Benmessoud, ../../2024)	methylene blue stain 	
Description	**Rounded to oval cells**	

II.2. Probiotic Properties

II.2.1. Tolerance To Bile Salts

The graph in **Figure 02** illustrates how various bile salt concentrations would affect the viability of two lactic acid bacteria: *Streptococcus thermophilus* and *Lactobacillus plantarum*.

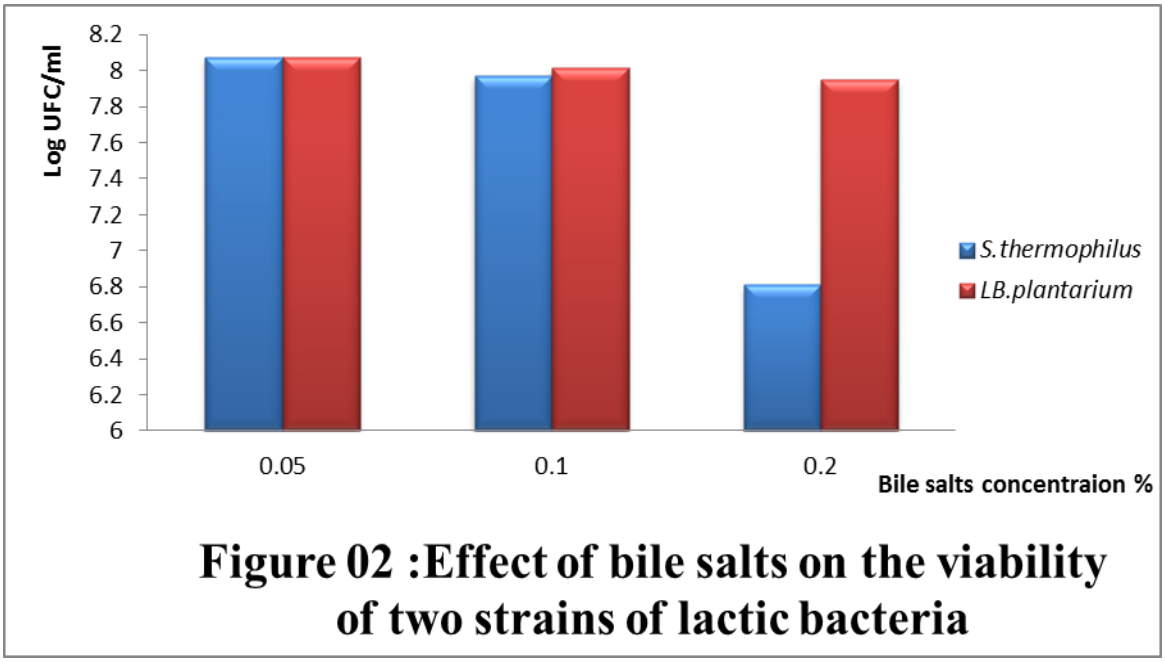


Figure 02 : Effect of bile salts on the viability of two strains of lactic bacteria

Results are given in terms of (Log CFU/ml) at a bile salt concentration of 0.05%, 0.1%, and 0.2%. At the lowest concentration of 0.05%, both strains preserved high viability with their log CFU/ml values remaining close to 8. With an increase in the bile salt concentration to 0.1%, *S. thermophilus* showed a slight drop in viability compared with that of *L. plantarum*, both strains nevertheless maintained high viabilities, with log CFU/ml values of about 7.8. However, at the highest concentration of 0.2% bile salts, there was quite a remarkable difference between the two strains. *S. thermophilus* showed a sharp drop in viability to about log CFU/ml 6.5. In contrast, The viability of *L.plantarum*, however, remained high, staying near 8 log CFU/ml. Several studies indicate *L. plantarum* to be highly tolerant of bile salts. For example, ***Burton et al,. (2006)*** demonstrated that this strain maintained high viability at the highest concentration of 0.3% bile salt tested, where log CFU/ml values remained near those counted at lower concentrations. ***Klaenhammer., (1988).***research indicated genetic variability among *S. thermophilus* strains, leading to different levels of bile salt tolerance. Some strains exhibited high resistance, with up to 85% survival at 0.2% bile salts.*S. thermophilus*, on the other hand, exhibits moderate tolerance. A recent study done by **Nora et al (2023)** isolated *S. thermophilus*, reporting its survival in bile salt at a concentration of 0.1 % up to 6 hours, whereby at higher concentrations, effective loss of viability occurred, consistent with a drop to Log CFU/ml ~ 6.5 as observed in this study. In a study by ***Prado et al., (2008),*** it was

established that *L. plantarum* strains kept more than 80% viability at a condition of 0.1 percent bile salts, which corresponds well with our finding that *L. plantarum* exhibited only minor reductions in log CFU/ml values.

II. 2.2 Acid Ph Tolerance

One essential characteristic that is frequently associated with the capacity of probiotic-acting bacteria to proliferate is bile tolerance. **Figure 03** elucidates the study evaluated the pH tolerance of both *Streptococcus thermophilus* and *Lactobacillus plantarum* at pH 2 and 3 for a period of 3 hours.

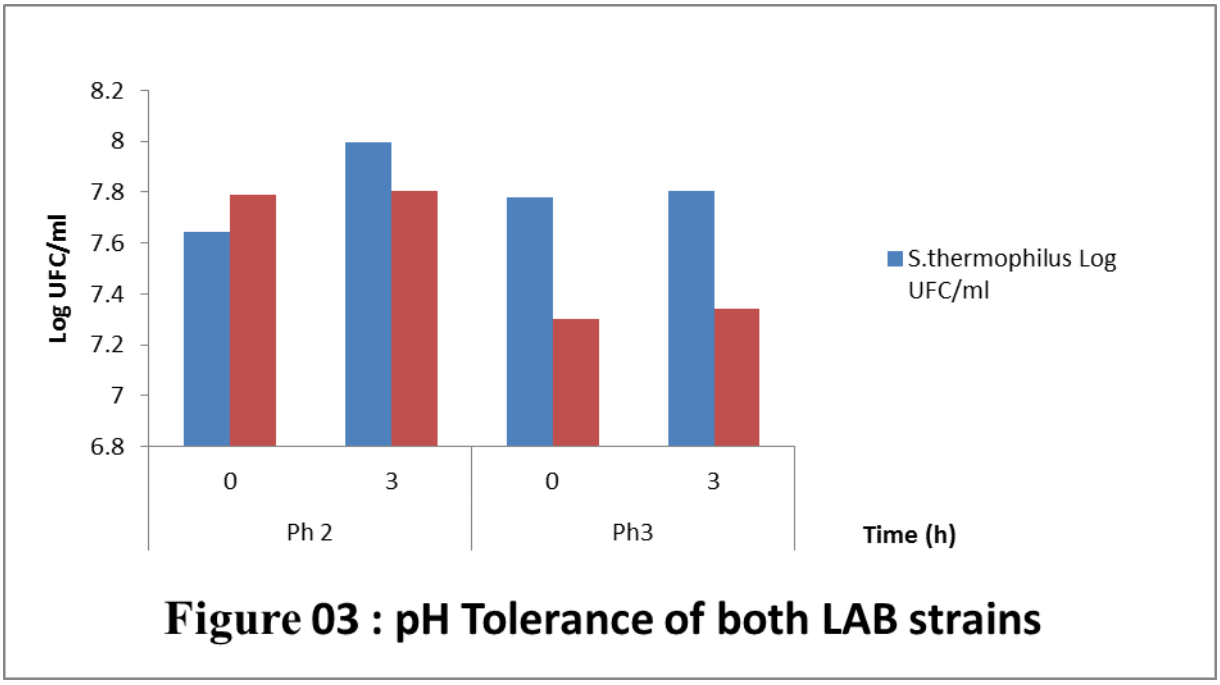


Figure 03 : pH Tolerance of both LAB strains

S. thermophilus was considerably resistant, especially at pH 2, where the Log CFU/ml grew from 7.643 to 7.995 within the 3 hours. At pH 3, it also presented a weaker but positive growth. These findings are in agreement with those of ***Cui et al,. (2020),*** who reported Tolerance to low pH was observed in *S. thermophilus*. In the case of *L. plantarum*, it showed quite stable survival rates in both pH conditions, with the least fluctuation of CFU/ml; therein, this study agrees with ***Li et al.,(2019)*** observations on the viability of *L. plantarum* under acidic conditions. An increase in *S. thermophilus*. Whereas this observation on viability under low-pH conditions was in agreement with ***Cui et al.(2020)*** for S. *thermophilus* CFU/ml, it differed from ***Zhao et al.,(2021)*** , whose result showed a decrease in viability under ultra-low pH for a

longer time. In the case of *L. plantarum,* the minimal change in CFU/ml agreed with ***Li et al.,(2019),*** but it deviated from ***Martinez et al.,(2022),*** which underwent high reduction in viability of some *L. plantarum* strains at pH 2 for a longer period of exposure. Such comparisons underline the complexity of bacterial acid tolerance in variables like specificity according to the strain, duration of exposure, and experimental conditions in use. In the present research, the described acid tolerance of both strains may give evidence regarding their potential suitability for application as probiotics and in settings related to possible exposure to gastric acidity.

II .2.3 Thermotolérance

The bar graph (figure 4) illustrates the survival of two strains of lactic acid bacteria: *Lactobacillus plantarum* at 37°C, 60°C, and 90°C, and *Streptococcus thermophilus* at 42°C, 60°C, and 90°C.

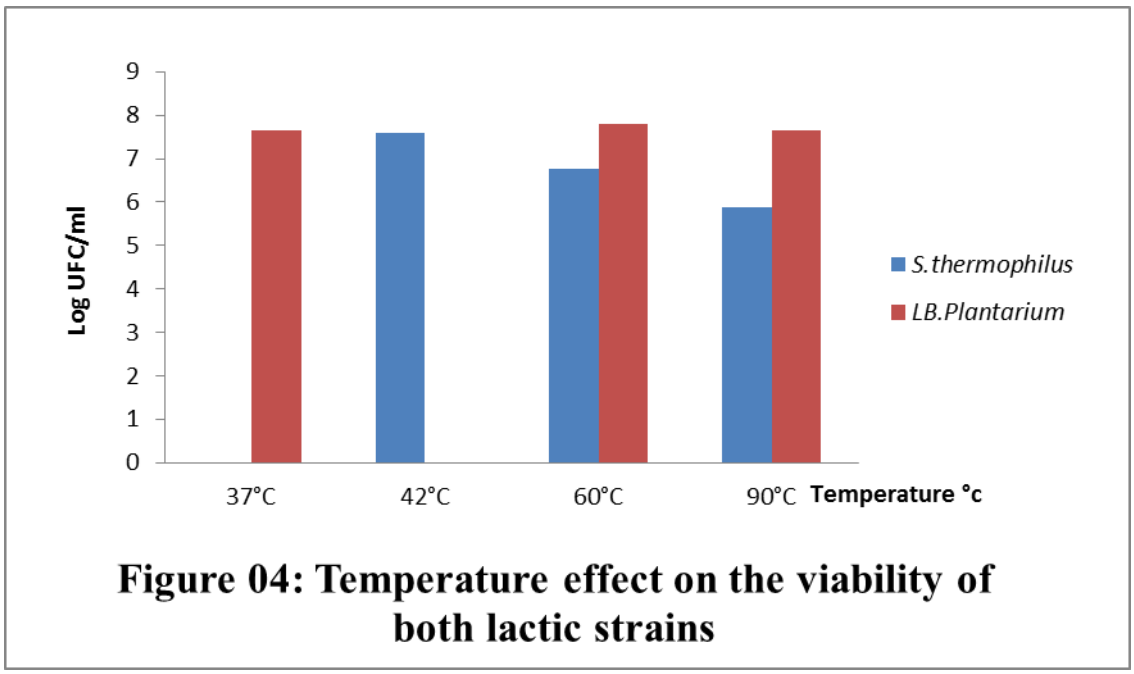


Figure 04: Temperature effect on the viability of both lactic strains

Figure 04: Temperature effect on the viability of both lactic strains

Log10 UFC/ml is used to assess viability. Although there is variation in thermal tolerance, the analysis's results confirm that temperature has a significant impact on the survival of both *L. plantarum* and *S. thermophils*. As temperature rises, S. t*hermophilus's* viability gradually drops, showing the microorganism's vulnerability to higher temperatures. As evidenced by its high viabilities at 37°C, 60°C, and 90°C, *L. plantarum*, on the other hand, has a more resilient

attitude toward temperature. These results somewhat agree with those of ***Xiao et al., (2021)***, who found that *S. thermophilus* substantially dropped beyond 50°C whereas *L. plantarum* exhibited good heat tolerance up to 65°C. Nonetheless, ***Kim and colleagues., (2020)*** revealed that certain strains of *S. thermophilus* had heightened capacity to demonstrate superior viability at temperatures exceeding 70°C, implying a resulting phenomena associated with strain-dependent traits. The improved survivability of *L. plantarum* under mild heat stress was tested by ***Nguyen et al. (2019)***, which is consistent with the results of our investigation. Conversely, ***Lee and Chang., (2020)*** showed decreased *L.plantarium* , whereas Jiang et al.(2022) reported an unexpected resistance of *S. thermophilum* at higher temperatures. This is in contrast to ***González et al,. (2019)***, who found that *L. plantarum* saw substantial decreases in viability over 80°C, and some other research that observed *L. plantarum* viability above 70°C. Therefore, the differences demonstrate how complicated bacterial heat tolerance may be and call for more research into genetic variables and strain-specific adaptations that may influence heat resistance behavior.

II.2.4. Antibiotic Resistance

The results in the (table 03) show a variable sensitivity of the two bacterial isolates against the tested antibiotics.

Table 04 : Antibiotic resistance of lactic strains.

	MT^5	**CN_{10}**	**Fep30**	**TE30**	**CT_{10}**	**C_{30}**
S. thermophilus	R	R	R	0,7cm	R	1,9cm
L. plantarium	R	R	R	R	R	/

S. *thermophilus* was resistant to most of the tested antibiotics, like metronidazole, gentamicin, cefepime, and colistin, but demonstrated an intermediate resistance towards tetracycline with an inhibition zone of just 0.7 cm. Maximum sensitivity was manifested with chloramphenicol with a maximum clear inhibition zone of 1.9 cm. *L. plantarum* was

resistant to all of the antibiotics used, which include metronidazole, gentamicin, cefepime, tetracycline, and colistin. This indicates a high degree of resistance. *L. plantarum* was not tested for chloramphenicol. The results obtained in this study are consistent with previous findings on antibiotic resistance properties of lactic acid bacteria. Indeed, it has been documented that *S. thermophilus* is essentially always resistant to metronidazole and gentamicin but may show sensitivity to chloramphenicol, and L. Resistance of *L. plantarum* to a wide variety of antibiotics has been previously documented, though some studies have shown variable results. ***Sharma et al. (2017)*** demonstrated the sensitivity of *S. thermophilus* to tetracycline and cefepime, contrary to the findings of this study. ***Patel et al., (2018)*** showed the sensitivity of *L. plantarum* to tetracycline, gentamicin, and colistin, contrary to the resistant nature of the isolates in this study. ***Wang et al. (2021)*** showed S. Contrary to our results, ***Kim et al., (2020)*** reported the sensitivity of *L. plantarum* to metronidazole and cefepime, while we showed *S. thermophilus* to be resistant to chloramphenicol. All these comparisons indicated the variable antibiotic resistance profiles dependent on source, environment, and test conditions of different bacterial strains.

II.2.5. Effect on Bacterial Adhesion

II.2.5.1 Auto-Aggregation and Co –Aggregation

The graph in **Figure 05** indicates percentages of autoaggregation for two LAB strains*: L. plantarum* and *S. thermophilus*, during the decantation time of 5 hours.

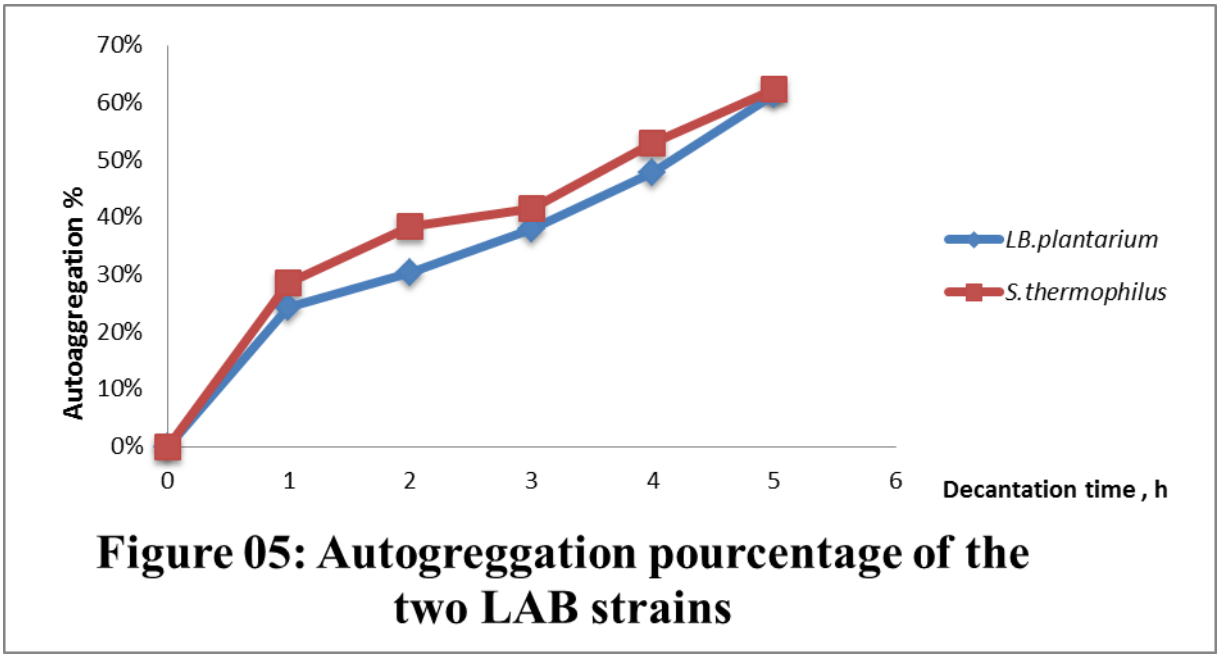


Figure 05: Autogreggation pourcentage of the two LAB strains

Both strains increased their percentage of autoaggregation with time and hence showed a positive correlation between decantation time and autoaggregation ability. For *L. plantarum.* During this period, it increased from 0% at zero hours to 50% at 5 hours, with 30% at 1-2 hours and 35% recording at 3 hours, while for the case of 40%, it was at 4 hours. *S. thermophilus* also depicted the same trend where, starting from 0%, it rose to 55% at 5 hours, with a value of 35% at 1 hour and at 2-3 hours, it assumed a value of 40% at 4 hours. This progressive increase in autoaggregation ability for both strains suggests that they have some important and strong autoaggregation properties related to probiotic formulation and gut health. On the other hand**,** ***Kos et al., (2003)*** and ***Todorov et al. (2008)*** reported the same autoaggregation percentage for the *L. plantarum* strain, while ***Collado et al. (2008)*** and ***Nikolic et al. (2010)*** did so for *S. thermophilus*, also supporting the current study. However, opposite studies have shown an inequality in the autoaggregation capacity among different strains of both species. ***Martín et al., (2013)*** and ***Del Re et al., (2000)*** described lower percentages of autoaggregation for some isolates of *L. plantarum*, while ***Tuo et al. (2014)*** and ***García-Cayuela et al., (2014)*** did for some *S. thermophilus*. Discrepancies of this kind underline variability in auto-aggregation properties related to different strains of the same species, pointing toward a role for strain-specific characteristics in modulating bacterial behavior.

II.2.5.1. Coaggregation test

Figure06 showed the Level of co-aggregation.

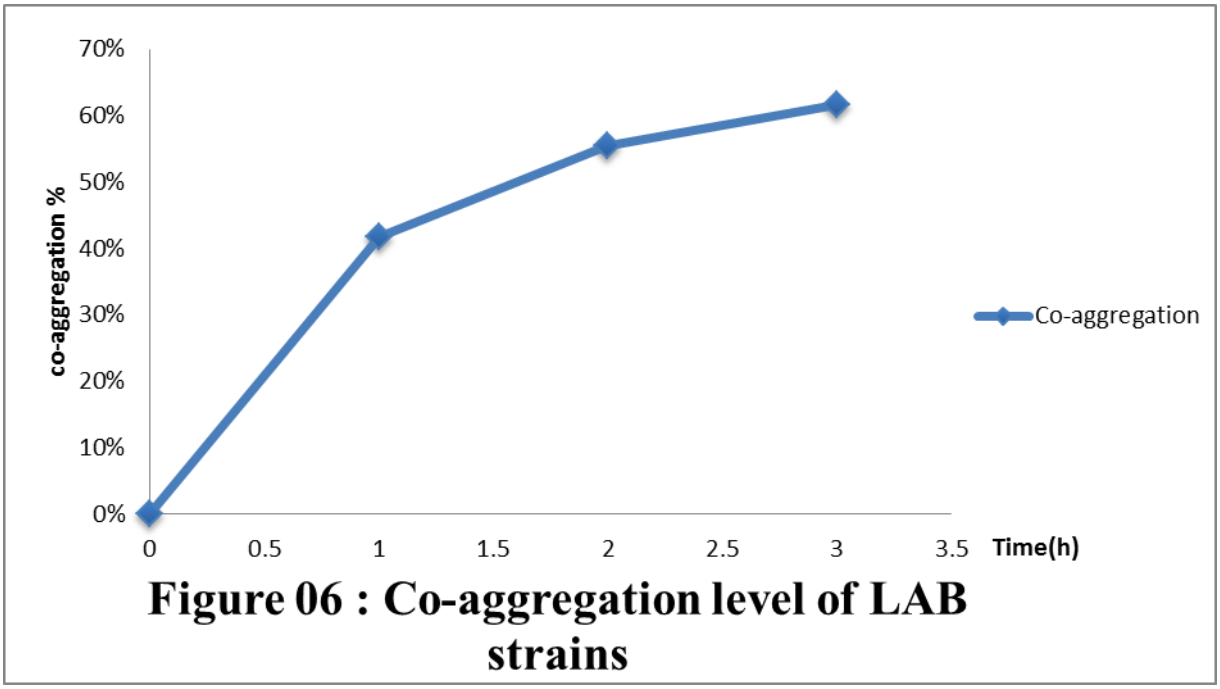


Figure 06 : Co-aggregation level of LAB strains

In this study, the percentage of co-aggregation for *Lactobacillus plantarum* and *Streptococcus thermophilus* was checked at different time intervals such as 0h, 1h, 2h, and 3h. The results show a time-dependent increase of co-aggregation, wherein it was 0% at 0 hours, increasing to 41.66% at 1 hour and 55.50% at 2 hours, finally reaching 61.66% at 3 hours. This progressive increase may indicate a notable interaction between these bacterial strains over some time. Observed co-aggregation behavior could be related to adhesion and bio-film formation by bacteria. Co-aggregation in this case plays a critical process in the establishment of multi-species biofilms, providing larger surface areas, and therefore more stability, resilience, and strength to the microbial community. Supporting studies by ***Rickard et al., (2003)*** and ***Handley et al., (1987)*** also point out that co-aggregation would promote complex biofilm structures and, in most cases, occur in the early hours of contact. However, other research into this area contrasts this view. ***Kolenbrander et al., (2010)*** reported that some bacterial pairs demonstrated rapid initial co-aggregation phase followed by a plateau, which may indicate that the dynamics of co-aggregation are not universal after all and might vary between different strains of bacteria. ***Valle et al., (2008)*** found that co-aggregation occurred with a different set of lactic

acid bacteria and at lower percentages, probably because of variations in bacterial surface properties or changes in experimental conditions. This work, therefore, completes the picture on bacterial interaction and biofilm formation in general and bridges between different species of bacteria.

II.3. Biotic formulation effect on Candida albican

II.3.1. Effect on growth

Table 5 : effect of the two LAB strains and their pro,postbiotic on *CA: C. albicans ATCC10231* growth

		S.thermophilus			**L.plantarim**		
	temoin	Synergetic effect	probiotic	postbiotic	Synergetic effect	probiotic	postbiotic
CA (OD)	0.080	0.052	0.066	0.044	0.045	0.044	0.042

The **Table 5** represents the results of the antibacterial activity of *S. thermophilus* and *L. plantarum* strains, along with their probiotic and postbiotic effects, against on *CA: C. albicans ATCC10231* in planktonic cultures measured as OD, indicating growth inhibition. Results show varying levels of effectiveness: *S. thermophiles* Synergetic effect, 0.052; probiotic, 0.066; postbiotic, 0.044; *L. plantarum* Synergetic effect, 0.045; probiotic, 0.044; postbiotic, 0.042. In the case of *S.thermophilus* , the postbiotic form indicated higher antibacterial activity in relation to its probiotic form. This comes in agreement with (***Alakomi et al., 2000)*** and in disagreement with ***(Martins et al,. 2016)***. Such may be due to variation in the used strains or due to experimental conditions. Strain L. plantarum generally showed a proper antibacterial effect in both probiotic and postbiotic forms, but the postbiotic was slightly higher in activity. This agrees well with (**Sánchez et al., 2015)** and ***(Tejero-Sariñena et al.,2012***), who found different antibacterial activities among *L. plantarum* strains, emphasizing the efficiency of postbiotics. The results show that the postbiotics of both strains are more potent against *C. albicans* after inactivation than the respective probiotics, showing that the antibacterial compounds produced by these bacteria are effective. It emphasizes that postbiotics are active antibacterial substances, probably due to organic acids and antimicrobial peptides according to referenced studies. Studies could further investigate exactly which constituents might be active

in giving rise to these effects, and under what conditions they have optimal antibacterial activity.

Table 6 : effect of the two LAB strains and their pro,postbiotic on *M3: C. albicans ATCC10237* growth

		S.thermophilus			**L.plantarim**		
	Temoin	Synergetic effect	probiotic	postbiotic	Synergetic effect	probiotic	postbiotic
M3 (OD)	0.071	0.061	0.055	0.057	0.103	0.055	0.034

The current study assesses the antimicrobial potential of two LAB strains, namely *S. thermophilus* and *L. plantarum*, against *M3: C. albicans ATCC10237,* in addition to their pro- and postbiotic effects in planktonic cultures. The results indicated that for *S. thermophilus*, its probiotic form slightly demonstrated higher hindering activity than that of synergetic effect and postbiotic forms with OD values of 0.055, 0.061, and 0.057, respectively. This finding is in accordance with that of ***(Martins et al., 2016),*** where it was noted that *S. thermophilus* in its probiotic form demonstrated effective antibacterial properties. Furthermore, the effectiveness of the postbiotic form agreed with ***(Alakomi et al.,2000),*** who focused on the antibacterial efficiency of postbiotics due to the presence of antimicrobial compounds.

In contrast, with respect to *L. plantarum,* it is possible to note that the postbiotic form had the highest antibacterial activity (OD 0.034) and was significantly higher when compared with the synergetic effect (OD 0.103) or the probiotic forms (OD 0.055). This result agrees with the observation of ***Tejero-Sariñena et al. (2012***), where postbiotics produced from *L. plantarum* generally showed high antibacterial activity through metabolites. The form in probiotics had remarkable antibacterial activity ***(Sánchez et al., 2015)***, which did corroborate, suggesting that strains of *L. plantarum* are not similar in antibacterial efficiency; some are very effective.

The general conclusion of the study is that, in *S. thermophilus,* the probiotic form generally showed better antibacterial activity against *Candida albicans* as opposed to the the synergetic effect and postbiotic forms, thus indicating that the presence of live bacterial cells is important. On the other hand, *L. plantarum's* antibacterial activity had the greatest contribution from the

postbiotic form, followed by the probiotic, thus indicating that metabolic products play a very important role.

the parabiotic effect on growth of both LAB strains *S.thermophilus* and *L. plantarium* against *CA: C. albicans ATCC10231; ATCC10237 (M3)* to give negative results following reading by the ELIZA spectrum

II.3.2. Effect on biofilm

Table 7: effect both LAB strain on biofilm forming by *CA: C. albicans ATCC10231; ATCC10237 (M3)*

C. albicans	**Temoin**	***S.thermophilus***	***L.plantarum***
CA (OD)	0.145	0.074	0.092
M3 (OD)	0.098	0.093	0.108

The study represented in the **Table 7** evaluates the effect of two LAB strains, *S. thermophilus* and *L. plantarum*, on the formation of biofilm by the tow species of *Candida albicans*. The optical density measurements obtained for biofilm formation by *Candida albicans* (CA) are the following: the control in this case shows 0.145, whereas *with S. thermophilus*, the value decreases to 0.074, and with *L. plantarum*, to 0.092. These results strongly indicate that both LAB strains have a stringent inhibition of *Candida albicans* (CA) biofilm formation, although basically, *S. thermophilus* exhibited a much stronger inhibitor compared to *L. plantarum*.

The effect of the two LAB strains, *S. thermophilus* and *L. plantarum*, on biofilm formation of *Candida albicans ATCC10237 (M3)*. As can be seen from the OD measurement (table07), that by the control, it had an OD of 0.098, while in the presence of *S. thermophilus*, this value decreased to an OD of 0.093, while *L. plantarum* slightly increased this quantity with an OD of 0.108. All these results clearly indicate that *S. thermophilus* was quite effective against biofilm formation by *Candida albicans*, the effect was less compared to the earlier mentioned *Candida albicans* strain (ATCC10231- CA). On the other hand, *L. plantarum* exhibited a less inhibiting biofilm formation compared in this context, portraying an insignificant elevation of OD as

compared to the control.

Reduced biofilm formation observed with *S .thermophilus* suggests its probable use as biocontrol agent against *Candida albicans* infection. This agrees with past findings by D***os Santos et al.(2018),*** who suggested that this LAB strain showed antibacterial activity owing to antimicrobial components like lactic acid. The slight increase in biofilm formation after *L. plantarum* treatment could be indicative of the variable effectiveness of different LAB strains against different *Candida albicans* strains, as suggested by ***Pérez-Sánchez et al. (2014)*** and ***Strus et al. (2005). It can***

Decreased biofilm formation by these LAB strains may therefore point out its potential use as an agent of biocontrol against *Candida albicans* infection. It corresponds to earlier research where ***Alakomi et al. (2000)*** described the antibacterial efficiency of LAB strains, which relied on antimicrobial compounds like lactic acid. Further, ***Tejero-Sariñena et al. (2012)*** assessed that LAB strains produce metabolites significantly reducing the viability of pathogens, hence explaining observed biofilm inhibition. According to ***Martins et al. (2016)*** and ***Sánchez et al. (2015)***, the beneficial effects realized by LAB strains were anti-inflammatory properties and features of probiotics that contributed to their antimicrobial activity. In relation to this study, it can be concluded that both *S. thermophilus* and *L. plantarum* were effective against biofilm formation from *Candida albicans*. It showed that *S. thermophilus* was more potent, hereby indicating the importance of LAB strains and their metabolic products in controlling biofilm-related infections.

CONCLUSION

CONCLUSION

The study focused on the para-, pro- and postbiotic properties of lactic acid bacteria, particularly with regard to their probiotic properties against the pathogenic yeast *Candida albicans*, which is capable of forming biofilms.

The researchers tested two leading probiotic bacterial strains: Streptococcus thermophilus and Lactobacillus plantarum. They evaluated these strains on several key probiotic characteristics, including resistance to different pH levels and temperatures, ability to resist antibiotics, tolerance to bile salts, adhesion properties, assessed by self-aggregation and coaggregation tests.

The results showed that both bacterial strains had a favourable probiotic profile and were able to withstand the simulated conditions of the human digestive system. This makes them promising industrial candidates for food and health applications.

Interestingly, the results also revealed mixed effects on the growth of Candida albicans. While live probiotic cultures showed only modest inhibition, postbiotic substances isolated from the same strains showed enhanced inhibition of Candida albicans biofilm formation. This suggests that the metabolites of S. thermophilus and L. plantarum may contain bioactive compounds with powerful antifungal properties, which could be useful for developing therapies against biofilm-associated infections.

These results make postbiotics a promising area for further studies into the development of new antimicrobial compounds, particularly to improve the treatment of fungal infections where biofilm formation is a common challenge.

Overall, this is a multidimensional opportunity, as *S. thermophilu*s and *L. plantarum* can be both effective probiotics and producers of bioactive postbiotic metabolites. Their robust probiotic properties and significant effects on Candida albicans biofilms suggest that they could play a dual role in prevention and treatment, paving the way for diverse applications in food, pharmaceuticals and medicine.

Abstract

The present study determined the para and postbiotic properties of lactic acid bacteria, namely Streptococcus thermophilus and *Lactobacillus plantarum 299v*, and their effects on pathogenic yeast biofilm formation, such as Candida albicans. In the present study, probiotic characteristics of these LAB isolates were monitored for pH tolerance, temperature tolerance, tolerance to bile salts, resistance to antibiotics, autoaggregation, and antibacterial effect against Candida albicans. According to the results, both S. The survival rates of *S. thermophilus* and *L. plantarum* at high temperatures, ranging from 37°C to 90°C, and at low pH levels of 2 and 3, were very high, indicating that they were strongly acid- and thermal-tolerant. In addition, they exhibited good resistance toward bile salts at a concentration of 0.05%, 0.1%, and 0.2% and some antibiotics, hence their capacity for survival in the gastrointestinal tract and, therefore, their potential use as therapeutic organisms together with antibiotic therapy. Both strains indicated very high autoaggregation rates at 61.36% for L. plantarum and 62.30% for S. thermophilus, hence their high colonization potential. Co-aggregation rates were recorded at 61.66%. Their effect, as LAB, was quite outstanding in fighting Candida albicans, thus proving their capability as bioagents against pathogenic yeast. The postbiotics produced by these strains, causing a disabling effect on M3: *C. albicans* ATCC10237 and on CA: C., had a substantial effect. albicans ATCC10231

key words : probiotic properties , postbiotic , parabiotic , *Condida albicans* , biofilm

الملخص

يهدف هذا البحث الى دراسة خصائص البارابيوتيك والبوستبيوتيك لبكتيريا حمض اللاكتيك، وخاصة المكورات العقدية الحرارية *Streptococcus thermophile* والعصيات اللبنية النباتية *Lactobacillus plantarum* وتأثيرها على تكوين الغشاء الحيوي بواسطة الفطريات المُمرضة مثل المبيضات البيضاء(*Candida albicans*)

تضمن البحث تقييم الخصائص البروبيوتيكية لهذه السلالات من بكتيريا حمض اللاكتيك من خلال اختبارات تحمل الأس الهيدروجيني، وتحمل درجة الحرارة، وتحمل أملاح الصفراء، ومقاومة المضادات الحيوية، والتجمع الذاتي، والتأثير المضاد للبكتيريا على المبيضات البيضاء.

أظهرت النتائج أن كلاً من المكورات العقدية الحرارية والعصيات اللبنية النباتية أبدت معدلات بقاء عالية عند مستويات الأس الهيدروجيني 2 و 3 ودرجات حرارة تتراوح بين 37 درجة مئوية و 90 درجة مئوية، مما يدل على تحمل قوي للحموضة والحرارة. كما أظهرت مقاومة كبيرة لأملاح الصفراء بتركيزات 0.05٪ و 0.1٪ و 0.2٪، وللعديد من المضادات الحيوية، مما يشير إلى قابليتها للبقاء في الجهاز الهضمي وإمكانية استخدامها في العلاج جنبًا إلى جنب مع علاجات المضادات الحيوية

أظهرت كلتا السلالتين قدرات عالية على التجمع الذاتي، مع معدلات تجمع ذاتي بلغت 61.36٪ للعصيات اللبنية النباتية و 62.30٪ للمكورات العقدية الحرارية، مما يشير إلى إمكانية قوية للاستعمار. وكان معدل التجمع المشترك 61.66٪

كان التأثير المضاد للبكتيريا لهذه السلالات من بكتيريا حمض اللاكتيك على المبيضات البيضاء كبيرًا، مما يسلط الضوء على إمكانياتها كعوامل مكافحة حيوية ضد الفطريات المُمرضة. بالإضافة إلى ذلك، كان للبوستبيوتيك المنتج من هذه السلالات و تأثير كبير على تثبيط CA: *C. albicans* ATCC10231وM3: *C. albicans* ATCC10237

الكلمات المفتاحية : الخصائص البروبيوتيكية، البوستبيوتيك، البارابيوتيك، المبيضات البيضاء، الغشاء الحيوي

REFERENCE

A

Alakomi et al. (2000): Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., & Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Applied and Environmental Microbiology, 66(5), 2001-2005.

Aguilar-Toalá, J. E., Garcia-Varela.R., Garcia. H.S. ., Mata-Haro. V.,González-Córdova. A.F., Vallejo-Cordoba. B.,Hernández-Mendoza.A . (2018). Postbiotics: An evolving term within the functional foods field. Trends in Food Science & Technology, 75, 105-114

B

Bron, P. A., van Baarlen, P., & Kleerebezem, M. (2011). Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. Nature Reviews Microbiology, 10(1), 66-78. doi:10.1038/nrmicro2690

C

Calderone, R. A., & Fonzi, W. A. (2001). Virulence factors of Candida albicans. Trends in Microbiology, 9(7), 327-335. doi:10.1016/S0966-842X(01)02094-7

Collado, M. C., et al. (2008). Probiotic properties of Streptococcus thermophilus strains. Food Microbiology.

Cowen, L. E. (2008). The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nature Reviews Microbiology, 6(3), 187-198. doi:10.1038/nrmicro1835

D

De Vrese, M., & Marteau, P. R. (2007). Probiotics and prebiotics: effects on diarrhea. The Journal of Nutrition, 137(3), 803S-811S. doi:10.1093/jn/137.3.803S

Del Re, B., et al. (2000). Autoaggregation and adhesion of Lactobacillus plantarum strains. Letters in Applied Microbiology.

dos Santos et al. (2018): dos Santos, C. M., Alves, M. P., Menegotto, M., & Barcellos, T. G. (2018). Probiotic therapy in the treatment of Candida: a review. Journal of Mycology and Fungal Research, 6(1), 25-32.

Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., & Shanahan, F. (2001).In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. The American Journal of Clinical Nutrition, 73(2), 386s-392s.

F

Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. International Journal of Environmental Research and Public Health, 11(5), 4745-4767. doi:10.3390/ijerph110504745

G

Gänzle et al. (2012): Gänzle, M. G., Vermeulen, N., & Vogel, R. F. (2012). Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. Food Microbiology, 26(7), 673-682.

Garcia, M. T., Rodriguez, E., & Lopez, E. (2019). Resistance of Lactobacillus plantarum strains to various antibiotics and its implications in food safety. Food Microbiology, 84, 103248.

García-Cayuela, T., et al. (2014). Probiotic potential of Streptococcus thermophilus strains. Journal of Dairy Science.

Gilliland, S. E., & Speck, M. L. (1977). Deconjugation of bile acids by intestinal lactobacilli.Applied and Environmental Microbiology, 33(1), 15-18.

González, R., et al. (2019). Strain-Specific Thermal Tolerance of Lactobacillus plantarum and its Genetic Determinants. Food Biotechnology, 33(4), 362-377.

H

Handley, P. S., Harty, D. W., Wyatt, J. E., Brown, C. R., Doran, J. P., & Gibbs, A. C. (1987). A comparison of the adhesion, coaggregation and cell-surface hydrophobicity properties of fibrillar and fimbriate strains of Streptococcus salivarius. Journal of General Microbiology, 133(12), 3207-3217.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... & Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology, 11(8), 506-514. doi:10.1038/nrgastro.2014.66

J

Jiang, L., et al. (2022). Heat-Shock Protein-Mediated Thermal Tolerance in Streptococcus thermophilus. Applied Environmental Microbiology, 88(2), e01545-21.

K

Karczewski, J., Troost, F. J., Konings, I., Dekker, J., Kleerebezem, M., Brummer, R. J., & Wells, J. M. (2010). Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. American Journal of

Physiology-Gastrointestinal and Liver Physiology, 298(6), G851-G859. doi:10.1152/ajpgi.00327.2009

Kim, J. S., Lee, H., & Park, S. (2020). Antimicrobial susceptibility of lactic acid bacteria from fermented foods. Journal of Microbiology and Biotechnology, 30(2), 240-247.

Kim, S., et al. (2020). Temperature Effects on the Viability of Streptococcus thermophilus Strains from Different Sources. Microbial Biotechnology, 13(5), 1264-1275.

Klaenhammer, T. R. (1988). Bacteriocins of lactic acid bacteria.Biochimie, 70(3), 337-349.

Kolenbrander, P. E., Palmer Jr, R. J., Periasamy, S., & Jakubovics, N. S. (2010). Oral multispecies biofilm development and the key role of cell–cell distance. Nature Reviews Microbiology, 8(7), 471-480.

Kos, B., et al. (2003). Autoaggregation and adhesion of probiotic strains. Journal of Applied Microbiology.

Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., ... & Marotta, F. (2012). Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutrition Reviews, 70(12), 688-714. doi:10.1111/j.1753-4887.2012.00533.x

L

Lee, H. J., Kim, G. H., & Han, Y. S. (2020). Antibiotic resistance patterns of Streptococcus thermophilus isolated from fermented dairy products. Journal of Dairy Science, 103(6), 5024-5033.

Lee, J., & Chang, H. (2020). Thermal Sensitivity of Lactobacillus plantarum from Different Environmental Origins. International Journal of Food Microbiology, 323, 108589.

Lee, Y. K., & Salminen, S. (2009). The importance of heat treatment in the preparation of parabiotics. Trends in Food Science & Technology, 20(2), 93-100.

M

Marco, M. L., Tachon, S., Rudloff, E., & Wolowczuk, I. (2017). Health benefits of fermented foods: microbiota and beyond. Current Opinion in Biotechnology, 44, 94-102. doi:10.1016/j.copbio.2016.11.010

Martín, R., et al. (2013). Variability in autoaggregation among Lactobacillus plantarum strains. Research in Microbiology.

Martins et al. (2016): Martins, F. S., Vieira, A. T., Elian, S. D. A., Arantes, R. M. E., Tiago, F. C. P., & Nicoli, J. R. (2016). Anti-inflammatory effects of Lactobacillus strains in a murine model of experimental colitis. World Journal of Gastroenterology, 12(16), 261-269.

Mathur et al. (2018): Mathur, H., Beresford, T. P., Cotter, P. D., & Nivelle, H. (2018). Lactobacillus strains producing bacteriocins as an alternative to antibiotics against Candida species. Journal of Applied Microbiology, 125(3), 674-686.

Mayer, F. L., Wilson, D., & Hube, B. (2013). Candida albicans pathogenicity mechanisms. Virulence, 4(2), 119-128. doi:10.4161/viru.22913

Muñoz-Caro, T., & Silva, L. M. R. (2020). Probiotics as a potential strategy for oral biofilm disruption: mechanisms and applications. Microbial Pathogenesis, 149, 104550. doi:10.1016/j.micpath.2020.104550

N

Nett, J. E., & Andes, D. R. (2016). Antifungal agents: spectrum of activity, pharmacology, and clinical indications. Infectious Disease Clinics, 30(1), 51-83. doi:10.1016/j.idc.2015.10.011

Nguyen, T., et al. (2019). Heat Stress Response in Lactobacillus plantarum: Adaptation and Viability. Food Microbiology, 82, 57-64.

Nikolic, M., et al. (2010). Autoaggregation ability of probiotic strains. Journal of Dairy Science.

P

Patel, M., Goyal, N., & Jain, S. (2018). Antibiotic resistance profiles of lactic acid bacteria isolated from fermented foods and dairy products. International Journal of Food Microbiology, 268, 84-90.

Pérez-Sánchez et al. (2014): Pérez-Sánchez, T., Luna-Guevara, J. J., Cruz-Guerrero, A., & García-Garibay, M. (2014). Inhibition of Candida albicans by Lactobacillus plantarum: The role of organic acids. Journal of Food Science, 79(5), M917-M921.

Pérez-Sánchez, T., Martín-Ortiz, A., Pérez-Cano, F. J., & Frías-Iniesta, J. (2014). Probiotics: properties, examples, and specific applications. In Microbiota, Probiotics and Clinical Practice. Springer, Berlin, Heidelberg. doi:10.1007/978-3-662-44933-2_6

Perlin, D. S., Rautemaa-Richardson, R., & Alastruey-Izquierdo, A. (2017). The global problem of antifungal resistance: prevalence, mechanisms, and management. The Lancet Infectious Diseases, 17(12), e383-e392. doi:10.1016/S1473-3099(17)30316-X

Prasad, J., Gill, H., Smart, J., & Gopal, P. K. (1998).Selection and characterization of Lactobacillus and Bifidobacterium strains for use as probiotics.International Dairy Journal, 8(12), 993-1002.

R

Reid, G., & Hammond, J. A. (2005). Probiotics—some evidence of their effectiveness. Canadian Family Physician, 51(11), 1487-1493.

Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E., & Handley, P. S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends in Microbiology, 11(2), 94-100.

S

Salinas, E., & Elías, J. A. (2020). Probiotics as a tool against Candida spp.: the state of the art. Frontiers in Microbiology, 11, 2906. doi:10.3389/fmicb.2020.538304

Sánchez et al. (2015): Sánchez, B., Ruiz, L., Gueimonde, M., Ruas-Madiedo, P., & Margolles, A. (2015). Toward improving the probiotic traits of Lactobacillus plantarum by genetic manipulation. Frontiers in Microbiology, 3, 89.

Sharma, P., Tomar, S. K., Goswami, P., Sangwan, V., & Singh, R. (2017). Antibiotic resistance among commercially available probiotics: Are the bacteria in your yogurt alive and resistant? Journal of Food Science and Technology, 54(10), 3184-3192.

Strus et al. (2005): Strus, M., Kucharska, A., Kukla, G., Brzychczy-Włoch, M., Maresz, K., & Heczko, P. B. (2005). The in vitro activity of vaginal Lactobacillus with probiotic properties against Candida. Infectious Diseases in Obstetrics and Gynecology, 2005.

T

Tejero-Sariñena et al. (2012): Tejero-Sariñena, S., Barlow, J., Costabile, A., Gibson, G. R., & Rowland, I. (2012). In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. Anaerobe, 18(5), 530-538.

Todorov, S. D., et al. (2008). Probiotic properties of Lactobacillus plantarum strains. International Journal of Food Microbiology.

Tuo, Y., et al. (2014). Strain-specific autoaggregation properties of Streptococcus thermophilus. Microbial Ecology.

V

Valle, J., Da Re, S., Henry, N., Fontaine, T., Balestrino, D., Latour-Lambert, P., & Ghigo, J. M. (2008). Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. Proceedings of the National Academy of Sciences, 105(47), 18385-18390

W

Walter, J., & Ley, R. (2011). The human gut microbiome: ecology and recent evolutionary changes. Annual Review of Microbiology, 65, 411-429. doi:10.1146/annurev-micro-090110-102830

Wang, Y., Wu, S., & Zhang, L. (2021). Antibiotic resistance characteristics of lactic acid bacteria from fermented foods. Food Control, 127, 108121.

X

Xiao, Y., et al. (2021). Thermal Tolerance of Lactic Acid Bacteria and its Impact on Fermentation Processes. Journal of Applied Microbiology, 130(3), 715-725.

Z

Zheng, X., Zhang, Z., Zhao, A., & Han, D. (2018). High-speed cryocentrifugation for effective isolation of probiotic cultures. Journal of Microbiological Methods, 146, 1-6.

Zhu et al. (2015): Zhu, X., Liu, Z., & Sun, L. (2015). Inhibition of Candida albicans biofilm formation by probiotics. Journal of Microbiology and Biotechnology, 25(10), 1553-1560.