BENEDITO RODRIGUES DA SILVA NETO
(ORGANIZADOR)

PROJETOS INOVADORES E PRODUÇÃO INTELECTUAL NA MICROBIOLOGIA

Atena Editora
Ano 2020

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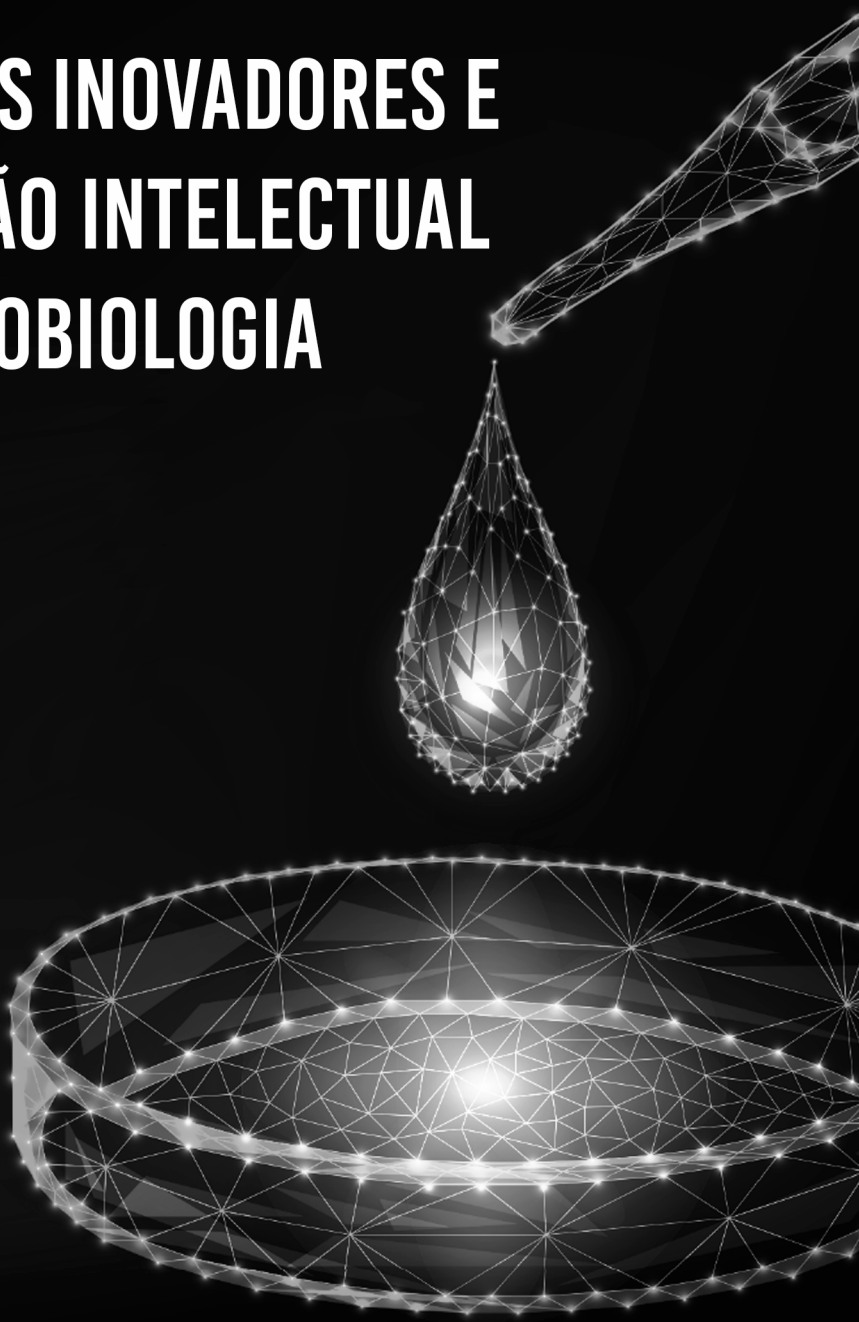

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Projetos inovadores e produção intelectual na microbiologia

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APRESENTAÇÃO

A microbiologia tem sido um assunto recorrente nos últimos anos, desde os corredores universitários aos locais informais, as conversas vão desde as bactérias multirresistentes, passando por novas espécies de fungos descobertos até chegar no atual momento de pandemia viral que marcará na história o ano de 2020. Esse campo de estudo amplo inclui o estudo dos seres vivos microscópicos nos seus mais vaiados aspectos como morfologia, estrutura, fisiologia, reprodução, genética, taxonomia, interação com outros organismos e com o ambiente além de aplicações biotecnológicas.

Como ciência, a microbiologia iniciou a cerca de duzentos anos atrás, e tem passado por constantes avanços graças a descobertas e inovações tecnológicas. Sabemos que os microrganismos são encontrados em praticamente todos os lugares, e a falta de conhecimento que havia antes da invenção do microscópio hoje não é mais um problema no estudo, principalmente das enfermidades relacionadas aos agentes como bactérias, vírus, fungos e protozoários.

A grande importância dessa temática se reflete no material de qualidade já publicado na Atena Editora e mais uma vez recebe os nossos holofotes com o tema “Projetos Inovadores e Produção Intelectual na Microbiologia” contendo trabalhos e pesquisas desenvolvidas em diversos institutos do território nacional contendo análises de processos biológicos embasados em células microbianas ou estudos científicos na fundamentação de atividades microbianas com capacidade de interferir nos processos de saúde/doença.

Temas ligados à inovação e tecnologia microbiana são, deste modo, discutidos aqui com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela saúde em seus aspectos microbiológicos. Deste modo, propomos aqui uma teoria bem fundamentada nos resultados práticos obtidos em diferentes campos da microbiologia, abrindo perspectivas futuras para os demais pesquisadores de outras subáreas da microbiologia.

Desejamos a todos uma excelente leitura!

Benedito Rodrigues da Silva Neto

SUMÁRIO

CAPÍTULO 14

PRODUCTION OF YEAST BIOMASS AND CELL WALL TO OBTAIN B GLUCANS FOR A BIOTECHNOLOGICAL PURPOSE

Data de aceite: 01/10/2020

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ABSTRACT: Considering that the composition of the yeast cell wall can vary with respect to different growth conditions, the objective of this study was to determine the influence of additives (EDTA, SDS, NaCl) on the production of yeast biomass, the percentage of cell wall and its composition, in order to obtain β-glucan. The extraction of the cell wall was carried out by an alkaline extraction technique and the study of the composition was carried out using infrared (IR) spectroscopy. The production of biomass and cell wall was variable, depending on the strain and the treatments studied. The IR spectra of each cell wall showed three characteristic regions of the wall (polysaccharides, proteins and lipids). In addition, it was possible to determine the chemical groups corresponding to β-glucan and amides I and II corresponding to chitin. The glucans content, and the β-glucan/chitin ratio in particular, varied with the yeast strain and with the additive studied. Future studies must be performed to extract the β-glucans and determine their concentration in each cell wall. The optimization of the production of cell wall and its components from yeast strains will allow its application in obtaining β-glucans for the food, pharmacological and cosmetic industries.

KEYWORDS: Additives, β-glucans, Cell Wall, Yeast.

PRODUÇÃO DE BIOMASSA DE LEVEDURA E PAREDE CELULAR PARA OBTER B GLUCANOS PARA UM PROPÓSITO BIOTECNOLÓGICO

RESUMO: Considerando que a composição da parede celular da levedura pode variar em função de diferentes condições de crescimento, o objetivo deste trabalho foi determinar a influência dos aditivos (EDTA, SDS, NaCl) na produção de biomassa de levedura, a porcentagem de parede celular e sua composição, a fim de obter β-glucano. A extração da parede celular foi realizada pela técnica de extração alcalina e o estudo da composição por espectroscopia de infravermelho (IV). A produção de biomassa e parede celular foi variável, dependendo da linhagem e dos tratamentos estudados. Os espectros de IV de cada parede celular mostraram três regiões características da parede (polissacarídeos, proteínas e lipídeos). Além disso, foi possível determinar os grupos químicos correspondentes à β-glucano e as amidas I e II correspondentes à quitina. O teor de glucanos, e a razão β-glucano/quitina em particular, variaram com a cepa de levedura e com o aditivo estudado. Estudos futuros devem ser realizados para extrair os β-glucanos e determinar sua concentração em cada parede celular. A otimização da produção

da parede celular e seus componentes a partir de cepas de leveduras permitirá sua aplicação na obtenção de β-glucanos para a indústria alimentícia, farmacológica e cosmética.
PALAVRAS - CHAVE: Aditivos, β-glucanos, Parede Celular, Levedura.

1 | INTRODUCTION

Yeasts are unicellular fungi that can be found in a variety of environment, approximately 900 species of yeast have been described, although the species studied in depth to be used in biotechnology are scarce. Among the most important yeast species, *Saccharomyces cerevisiae* is stands, which is the yeast most used in industrial processes. In recent years, other so-called non-conventional yeast species have become increasingly relevant due to their positive contribution to both food and beverage fermentation. Some of these yeast genera include *Pichia*, *Metschnikowia*, *Kluyveromyces* and *Issatchenkia* that participate in cider and wine fermentations, dairy products, bread production, sausages and various vegetable fermentations (Romano et al., 2006).

2 | YEAST BIOTECHNOLOGY

Biotechnology is any technological application that uses biological systems and living organisms or derivatives to make or modification of products and processes for specific uses. The biotechnological bases are the engineering, physics, chemistry, biology, human and veterinary medicine; and the field of this science has a great impact on pharmacy, medicine, food science, solid, liquid, gaseous waste treatment and agriculture (OECD, 2005).

Traditional yeast biotechnology has been carried out since the beginning of human history through the baking of bread, the preparation of alcoholic beverages and the cultivation of food or the raising of domestic animals. *Saccharomyces cerevisiae* has been described as humanity's most domesticated organism and is still the most exploited yeast species in the industry today. But recent developments in molecular biology have given biotechnology new meaning, new prominence, and new potential. (https://www.thebeertimes.com/saccharomyces-cerevisiae-y-las-claves-para-el-estudio-de-las-ciencias-de -life/).

Yeasts are the leading producer of biotech products worldwide, exceeding the production, capacity and economic income of any other group of industrial microorganisms. The global annual production of *S. cerevisiae* is over 1 million tons (Zymanczyk- Duda et al., 2017). Of all the yeast species studied, only a dozen is used on an industrial scale. On the other hand, around 70 to 80 laboratory scale species have been shown to have potential value in biotechnology (Deak, 2009; Kurtzman et al., 2011).

MAIN SPECIES OF BIOTECHNOLOGICAL IMPORTANCE	
Saccharomyces cerevisiae	*Scheffersomyces stipitis*
Schizosaccharomyces pombe	*Picchia spp.*
Kluyveromyces lactis	*Rhodotorula spp.*
Kluyveromyces marxianus	*Rhodosporidium spp.*
Schwanniomyces occidentalis	*Yarrowia lipolytica*
Lipomyces spp.	*Candida spp.*
Saccharomycopsis spp.	*Trichosporon spp.*
Debaromyces hansenii	*Blastobotryss adeninivornas*
Ogataea polymorpha	*Xanthophyllomyces dendrorhous*
Komagataella pastoris	

Boekhout (2003). The Yeasts. A Taxonomy Study. Kurtzman, Fell, Boekhout 2011.

3 | YEAST CELL WALL AND POLYSACCHARIDES

Yeast cells are surrounded by a rigid cell wall (CW) that provides physical and osmotic protection, as well as determining cell shape and integrity during cell growth and division; however, it is a dynamic structure that can adapt to physiological and morphological changes (Smith, 2000; Klis et al., 2002). This structure represents between 20 and 25% of the dry weight of the cell and consists of approximately 85 to 90% of polysaccharides and 10 to 15% of proteins (Walker, 2000).

Three different groups can be distinguished within the CW polysaccharides: on the one hand, mannose polymers or mannanoproteins that are a heterogeneous group of glycoproteins and can represent approximately 40% of the dry weight of the CW; on the other hand, the glucose polymers or β-glucan that constitute 60% of the dry weight of the CW, and can be differentiated, according to the type of glycosidic bonds, in β (1,3) and β (1,6) glucans, which represent 85% and 15% of these polysaccharides, respectively; and finally, the N-acetylglucosamine polymers that make up chitin and that constitute up to 2% of the dry weight of the CW (Nguyen et al., 1998; Klis et al., 2002; Aguilar-Uscanga and François, 2003; Kath and Kulicke, 1999; Suphantharika et al., 2003). The chemical structure of carbohydrates in the CW is shown in **Figure 1**.

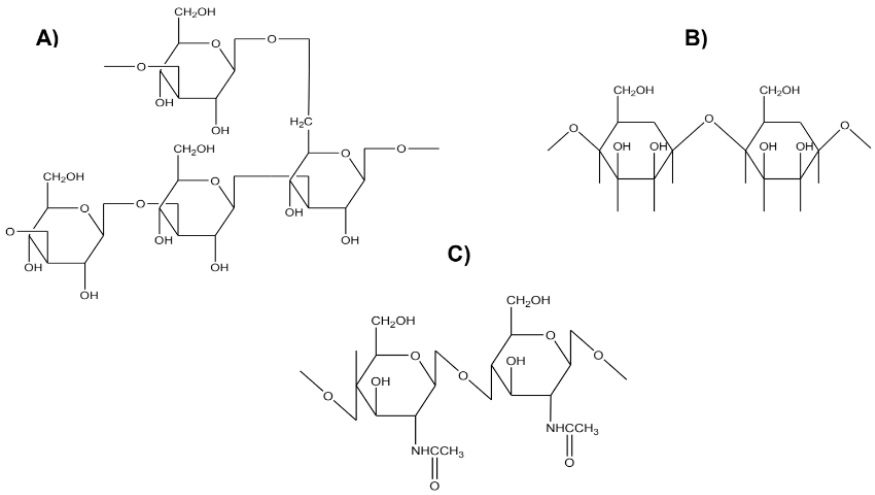

Figure 1. Chemical structure of polysaccharides from yeast cell walls. A) β glucans, B) Mannans and C) chitin.

Cell wall polysaccharides extracted from yeast have been described as components with important biological properties in addition to their structural function, as immunomodulators (Novak and Vetvicka, 2009; Mehdi and Hasan, 2012), with anti-inflammatory and antimicrobial (Vetvicka and Vetvickova 2010), antiproliferatives (Salvador et al., 2008) and antioxidants properties (Jaehrig, et al., 2007; Machová and Bystrick'y, 2013; Qiling et al., 2017). Furthermore, the physical and chemical properties of β-glucans and mannans have special interest over the years for different industrial applications (Zhu et al., 2015; Galineri et al., 2017). Studies have been carried out on the use of β glucans from different origins in the food industry (Kittisuban et al., 2014; Lazaridou et al., 2014; Rinaldi et al., 2015), in the cosmetic industry (Vacharaprechakul et al., 2007; Kanlayavattanakul and Lourith, 2008; Medeiros et al., 2013; Du et al., 2014) and in medicine (Belcarz et al., 2013; Samuelsen et al., 2014; Levitz, 2014).

4 I NUTRITIONAL FACTORS THAT INFLUENCE THE COMPOSITION OF THE YEAST CELL WALL

The composition of polysaccharides, the structure and the thickness of the cell wall vary according to the different species and strains, the stage of the cell cycle and environmental conditions (Nguyen et al., 1998; Aguilar-Uscanga and François, 2003; Bähler, 2005; Smits et al., 1999; Magnelli et al., 2002) (Table 1).

Knowledge about its composition and the factors that influence it is of relevance, due to the increased commercial interest in the production of β-glucans and mannans for the different industries (Donzis, 1996; Jozef et al., 1999; Zhu et al., 2015) and, conversely, the

need to reduce wall thickness to optimize a more effective and rapid release of endogenous biomolecules (Daran et al., 1997).

The most important factors for microbial growth are: the energy source and the carbon source and its use. The economic evaluation of the yeast production process suggests that the main contributor to the global cost is the carbon source. In recent years, different researchers have worked on obtaining CW and β-glucans using different carbon sources (**Table 1**). In biomass production, some criteria must be taken into account for the choice of the substrate. These are price, availability and abundance, toxicity, carbohydrate content and their use by microorganisms (Sharma et al., 2014).

FUNGI	SPECIES	CARBON SOURCE	REFERENCE
Yeast	*S. cerevisiae*	YPD (EDTA, SDS and ClNa)	Naruemon y col., 2013
	S. cerevisiae	YPD	Plata y col., 2013
	S. cerevisiae	Mineral medio (glucose, mannose, galactose, sucrose, maltose, ethanol)	Aguilar Uscanga y col. 2005
	S. cerevisiae, S. boulardi	YPD, DDGse (dried distiller grains with solubles extract)	Pereyra et al., 2018
	Kluyveromyces marxianus	YPD, DDGse (dried distiller grains with solubles extract)	Pereyra et al., 2017
Mushrooms	*Pleurotus sp.*	Olive mill solid waste (OMSW)	Avni et al., 2017
	Lentinus edodes	Olive mill waste (OMW) and phenols	Reverberi et al., 2004
	Lentinus edodes	Substrates with a high content of polyphenolic compounds	Ooi and Liu, 2000

Table 1. Use of different carbon sources and / or additives for obtaining cell wall and β glucans from fungi.

5 I MATERIALS AND METHODS

5.1 Yeast Isolation

Yeast strains were isolated from fifteen (15) samples of whole corn. Ten (10) grams of each sample were ground, weighed and added to 90 mL of sterile peptone water contained in an Erlenmeyer flask, obtaining a 10^{-1} dilution. Serial dilutions in sterile peptone water were made and 0.1 mL aliquots were inoculated in duplicate onto yeast dextrose extract (YPD) medium containing chloramphenicol (2 mL of a solution of 250 mg / 100 mL 95% ethanol). The plates were incubated at 28ºC for 24-48 h.

5.2 Molecular Identification Of Yeast Strains

Yeasts culture and DNA extraction

Yeast strains were maintained on plates containing YPD medium. Several colonies were transferred to a sterile microtube by duplicate. The pellet was frozen with liquid nitrogen for 5 min. Fungal DNA was extracted using a hexadecyltrimethylammonium bromide (CTAB 2%) (2% CTAB-Sigma; 1,4 M NaCl; 20 mM EDTA; 100 mM Tris-HCl 1M-pH 8) preheated to 65ºC and 10 μL of 2-mercaptoethanol (Merk) procedure following the methodology proposed by Leslie and Summerell (2006). Extraction residues were resuspended in 100 μL of 1X TE buffer (10mM Tris-HCl, 1.0mM EDTA, pH 8), and stored at -20°C. DNA quantification was performed using a Nanodrop 2000 Spectrophotometer kit (Thermo Scientific).

Obtaining profiles using PCR fingerprinting

Microsatellite-primed PCR. The one step PCR-fingerprinting method was performed using the microsatellite primer $(GTG)_5$. PCR reactions were made with 20-30 ng of fungal DNA in a total volume of 25 μL of 1 × reaction buffer containing 2 mM $MgCl_2$, 1.25 U Taq DNA polymerase (5 U.μL^{-1}, Invitrogen by Life Technologies, Buenos Aires, Argentina), 0.2 mM of each dNTP and 0.6 μM of GTG_5 primer. A negative control, containing all reagents without fungal DNA, was included in every set of reactions. PCR was conducted according to the following cyclic conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of 94°C for 45 s, 54°C for 45 s and 72°C for 1 min, and a final extension step of 72°C for 10 min, and then held at 4°C indefinitely. DNA band patterns were visualized after electrophoretic run on 1.5% agarose gel stained with 0.5 μg.mL^{-1} ethidium bromide and gels were photographed using a MiniBIS Pro, DNR Bioimaging systems analyzer. The fragment sizes were measured by comparison with DNA 100-bp ladder (Invitrogen by Life Technologies, Buenos Aires, Argentina) whose reference bands vary between 100 and 2072 bp. In addition, DNA profiles of *Saccharomyces* sp. and *Candida* sp. strains were used for comparison.

5.3 Biomass Production Of Yeast Strains To Obtain Cell Wall

For the biomass production, three yeast strains were selected that are beneficial according to the literature. The strains were maintained on malt extract agar (MEA).

Yeast biomass production

A concentration of 1.10^7 cells.mL^{-1} (1 mL) of yeast was inoculated into *Erlenmeyer*-type flasks containing 100 mL of YPD broth at 28°C for 24 h in orbital shaker (150 rpm). At the end of the incubation time, the biomass was centrifuged at 5000 rpm for 5 min. The sediment was dried in a forced air oven to recover the biomass destined for extraction from the cell wall.

Influence of the addition of EDTA, SDS and ClNa

The influence of the additives was determined on: I) biomass production and the percentage of CW, II) the production of β glucans, and III) and the β glucan/chitin ratio in the CW of the studied strains.

YPD medium was adjusted to pH 4 and supplemented with different concentrations of EDTA, SDS and ClNa, according to Nareumon et al., 2013 (**Table 2**). Each medium was inoculated with 1 mL of a concentration of 1.10^7 cells.mL^{-1} of yeast and incubated at 28°C for 24 h in orbital shaker (150 rpm). At the end of the incubation time, the culture media were centrifuged. The sediment (biomass) was dried in a forced air oven to recover the biomass destined for the extraction of the CW. All tests and combinations were performed in duplicate.

Additives (ppm)	Treatments						
	T1 (YPD)	T2	T3	T4	T5	T6	T7
EDTA	0	0	50	0	5	0	5
SDS	0	100	0	0	10	20	10
ClNa	0	0	0	30.000	0	3000	3000

Table 2. Concentration of additives used in YPD medium.

Preparation of the yeast cell wall

Biomass production was performed following the methodology of Nguyen et al., 1998, with some modifications. The cultures were incubated at 28°C in an orbital shaker (150 rpm). Yeast cells were harvested by centrifugation at 5000 rpm for 10 min, washed three times with water to remove traces of culture medium and then twice more with 0.1 M sodium phosphate buffer, pH 8.5 (at 4°C). The biomass was dried at 60°C in forced air oven until constant weight.

Yeast cells were suspended in 0.1 M sodium phosphate buffer, pH 8.5, and an equal volume of glass beads (0.45 mm diameter). They were cooled to 4°C and broken by mechanical shaking for 30 s, after which the homogenate was cooled and disrupted for another 30 s. This procedure was repeated five times. The glass beads were removed from the homogenate by decanting, and the cell walls were separated by centrifugation at 5000 rpm for 15 min. The cell walls were washed five times with 0.1 M phosphate buffer, pH 8.5, and then washed a further four times with distilled water. The temperature was kept below 4°C during all operations. The CW was dried at 60°C in forced air oven until constant weight.

5.4 Study Of The Composition Of The Cell Wall

To verify the variation in the composition of the CW under the influence of the

different additives (EDTA, SDS, ClNa) infrared spectroscopy (IR) was performed. The dried CW was mixed with KCl (1 mg CW with 200 mg KCl). This mixture was ground in an agate mortar, finally making the tablet was performed under pressure (≈15 ton.cm^{-2}) and applying dynamic vacuum for 15 min. Measurements were performed in a Nicolet FTIR Impact 400 spectrometer. For data acquisition and processing the software OPUS was used. The spectra were the result of an accumulation of 200 measurements, to increase signal/noise ratio, and were measured between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

5.5 Statistic Analysis

Assays were performed in duplicate for each treatment. An analysis of variance (ANOVA) was performed. When the analysis was statistically significant, the Duncan test was used to determine the importance of each individual parameter and its interactions at p≤0.05. For the analysis of the data, the Info Stat program for Windows version 2015 was used.

6 I RESULTS

6.1 Isolation And Identification

Twenty-five (25) yeast strains were isolated from the 15 samples analyzed. DNA extraction and fingerprinting of all strains was performed. Two strains with the band profile of *Saccharomyces* sp. and one strain with the band profile similar to that obtained for *Candida* sp. were selected. In a future study, the sequencing of the strains will be carried out to determine specifically what species they are.

6.2 Biomass And Cell Wall Production

Table 3 shows the biomass production and the CW percentage of the studied strains. The biomass production was higher in treatment 4 (with ClNa) for the strain *Saccharomyces* sp. 1 (0.87 g/ 100 mL) and in treatment 5 (EDTA and SDS) for the remaining strains (0.86 g/ 100 mL for *Saccharomyces* sp. 2 and 0.79 g/ 100 mL for *Candida* sp.). The average percentage of CW ranged between 10.6 and 40.3% for *Saccharomyces* sp. 1, from 13.3 to 36.1% for *Saccharomyces* sp. 2, and from 27.4 to 40.5% for *Candida* sp. For *Saccharomyces* sp. 1, the percentage of CW was higher (40.3 - 39.1%) when it was grown only in YPD medium (T1) and in YPD with the 3 additives (T7). For *Saccharomyces* sp. 2 it was observed that in the T6 treatment (SDS - ClNa) it achieved the highest percentage of CW (36.1%) and for *Candida* sp. the highest percentage of CW was when it was grown in YPD medium with EDTA and SDS (T5).

Treatments	Strains					
	Saccharomyces sp. 1		*Saccharomyces* sp. 2		*Candida* sp.	
	Biomass production (g/100 mL)	CW percentage (%)	Biomass production (g/100 mL)	CW percentage (%)	Biomass production (g/100 mL)	CW percentage (%)
T1	0.67	40.3	0.69	32.8	0.75	36.0
T2	0.62	10.6	0.58	13.3	0.62	27.4
T3	0.69	34.8	0.62	29.0	0.68	30.9
T4	0.86	25.6	0.73	30.1	0.68	33.8
T5	0.75	36.0	0.86	31.4	0.79	40.5
T6	0.61	26.2	0.72	36.1	0.74	33.8
T7	0.68	39.1	0.54	29.6	0.75	32.8

Table 3. Biomass production and cell wall (CW) percentage of *Saccharomyces* sp. 1, *Saccharomyces* sp. 2 and *Candida* sp. obtained from the different treatments.

T1: Control (YPD), T2: SDS, T3: EDTA T4: ClNa, T5: EDTA – SDS, T6: SDS – ClNa, T7: EDTA – SDS – ClNa.

The correlation between the amount of biomass (g/ 100 mL) and the amount of CW (g/ 100 mL) was determined for the 3 studied strains (**Figure 2**). For the strain *Saccharomyces* sp. 1 there was no correlation between both parameters. For the *Saccharomyces* sp. 2 and *Candida* sp. a positive correlation was observed between cell concentration and WC content, with values of R= 0.83 and R= 0.94, respectively.

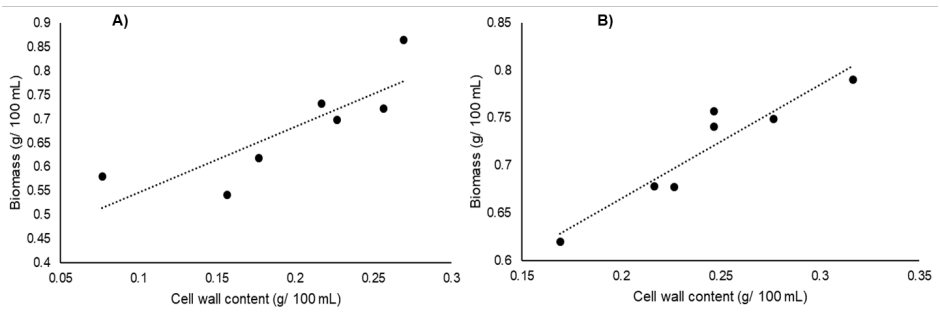

Figure 2. Correlation between cell wall content and amount of biomass. A) *Saccharomyces* sp. 2, B) *Candida* sp.

6.3 Study Of The Composition Of The Cell Wall

To verify the variation in the composition of the CW under the influence of different carbon sources, infrared (IR) spectroscopy was performed. The spectra of each CW in the 7 studied treatments show three regions corresponding to carbohydrates (950–1185 cm⁻

^{1}), proteins (1480–1700 cm^{-1}) and lipids (2840 - 3,000 cm^{-1}). In the carbohydrate region, the β-glucans are observed and, in the protein region, the chitin is shown, represented by the bands amide I and amide II (**Figures 3**, **4** and **5**). The 3 strains behaved differently in the 7 studied treatments. In addition to determine the presence of glucans and chitin, a semi quantitative comparison of the main infrared bands present in the 7 treatments for each strain was carried out to determine which of the additives present in the culture medium enhances the β-glucans production. **Figure 3** shows the infrared spectrum of the CW of the *Saccharomyces* sp. 1 strain where it showed a higher amount of carbohydrates (region between 1400 and 800 cm^{-1}) when it was cultivated in YPD medium with SDS (T2), in that treatment it is observed that the β glucans/chitin ratio is greater than 1. The combination of the three additives (EDTA, SDS, NaCl) in the culture medium (T7) also enhanced the production of β-glucans. **Figure 4** shows the infrared spectrum of the CW of the *Saccharomyces* sp. 2 strain. The T5 treatment (EDTA, SDS) was theoretically the best for obtaining carbohydrates, however, the β glucan/chitin ratio is of 1. By contrast, the treatment T2 (SDS) shows that the relation β glucans/chitin is greater than 1. The infrared spectrum of the CW of the *Candida* sp. strain is shown in **figure 5**. Treatments 1 and 2 are similar in terms of the influence on the production of β glucans. The β glucans/chitin relationship is 1 for all treatments, including T1 and T2.

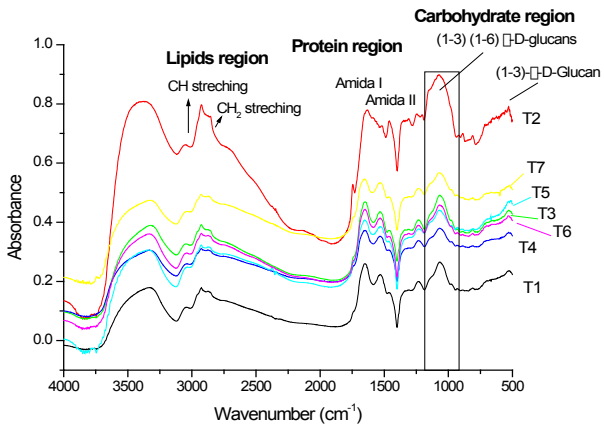

Figure 3. FITR spectra of *Saccharomyces* sp. 1 cell wall grown in different treatments. T1: Control (YPD), T2: SDS, T3: EDTA T4: ClNa, T5: EDTA – SDS, T6: SDS – ClNa, T7: EDTA – SDS – ClNa.

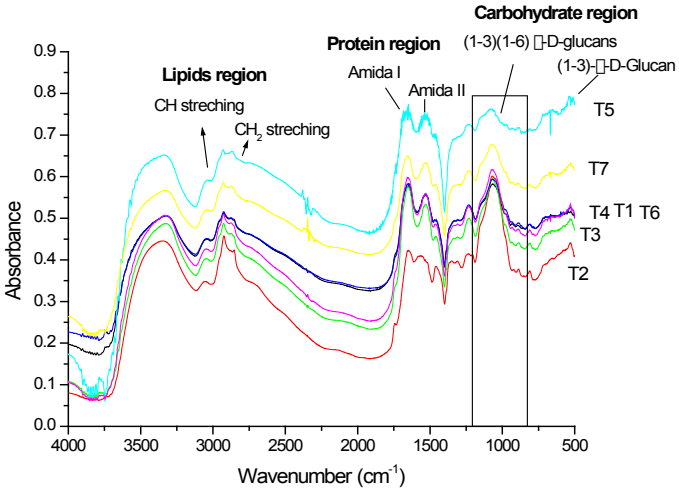

Figure 4. FITR spectra of *Saccharomyces* sp. 2 cell wall grown in different treatments. T1: Control (YPD), T2: SDS, T3: EDTA T4: ClNa, T5: EDTA – SDS, T6: SDS – ClNa, T7: EDTA – SDS – ClNa.

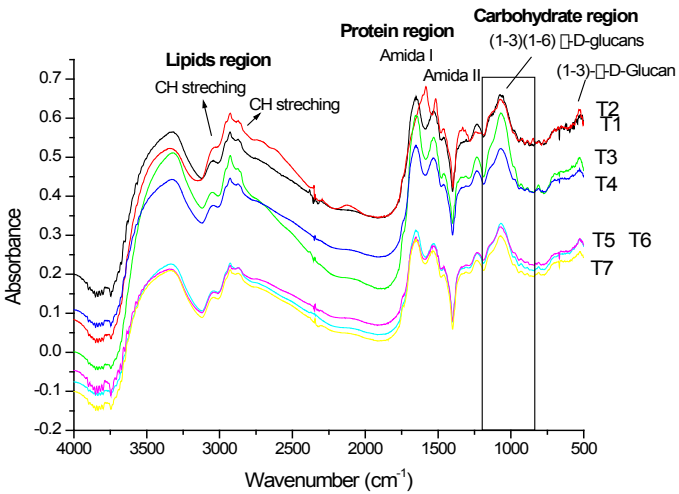

Figure 5. FITR spectra of *Candida* sp. cell wall grown in different treatments. T1: Control (YPD), T2: SDS, T3: EDTA T4: ClNa, T5: EDTA – SDS, T6: SDS – ClNa, T7: EDTA – SDS – ClNa.

7 | DISCUSION

This study evaluated the incorporation of 3 additives (EDTA, SDS and ClNa) to a culture medium such as YPD, to determine the influence on biomass production and cell wall content of *Saccharomyces* sp. 1; *Saccharomyces* sp. 2 and *Candida* sp. strains to be used in the future as producers of β-glucans.

The biomass concentration of the 3 studied strains in the 7 treatments was higher than those found by Serrat Díaz et al., (2017), they studied the influence of the carbon source, nitrogen source and temperature, where the average concentration ranged from 0.089 to 0.465 (g/ 100 mL). Dhanasekaran et al., (2011) studied the production of yeast biomass using pineapple residue, where a dry biomass content of 0.572 (g/ 100 mL) for *S. cerevisiae* and 0.492 (g/ 100 mL) for *C. tropicalis* was observed. Pereyra et al., (2018) studied the use of YPD medium and dried distiller grains with soluble extract (DDGse) for the biomass production of 3 probiotic strains (2 strains of *S. cerevisiae* and *S. boulardii*). These authors demonstrated a production between 0.405 to 0.5 (g/ 100 mL) in YPD and from 0.315 to 0.402 (g/ 100 mL) in DDGse. In this study the biomass production was used for the extraction of CW and to determine the content of β-glucans.

In general, the CW of *Saccharomyces cerevisiae*, is about 70 nm thickness that represent 20% of the whole cell's weight (Walker, 1999). The CW values found in this work varied between 10.6 to 40.3% (*Saccharomyces* sp. 1), from 13.3 to 36.1% (*Saccharomyces* sp. 2) and from 27.4 to 40.5% (*Candida* sp.). The average contents of CW were mostly within the range usually reported for yeast (Lipke and Ovalle, 1998). The composition of the CW can vary under different growth conditions, including the type of culture, carbon source, temperature, pH, and aeration (Aguilar-Uscanga and François, 2003; Naruemon et al., 2013). Aguilar Uscanga et al., (2005) studied the variation of polysaccharides composition present in the CW of *S. cerevisiae* using different carbon sources (glucose, mannose, galactose, sucrose, maltose, and ethanol) and found that the percentage of CW in dry weight was 10% for the culture made with 25% ethanol in the sucrose culture. Serrat Díaz et al., (2017) obtained CW percentages of *Kluyveromyces marxianus* from 12.3 to 27.6% when it was grown on different carbon sources (glucose, sucrose and lactose), different sources of nitrogens (peptone and $(NH_4)_2SO_4$)), different temperatures (30 and 40 ° C), pH (3.4 and 5.4) and in aerobiosis and microarobiosis. Nguyen et al., (1998) considered that it is important to maximize the performance of cell walls to minimize economic costs, if used commercially.

In this work, the composition of the extracted CW using FTIR was studied. The FTIR spectroscopy can be applied as a useful tool for the analysis of entire yeast cells providing a fast, effective, reagent-free, and simple method (Kuligowski et al., 2012). The FTIR spectroscopy is a rapid, precise, and accurate method, not requiring sample preparation, for the determination and quantification of carbohydrate composition of yeast (Novák and

Vetvi, 2009; Novák et al., 2012; Plata et al., 2013). The functional groups present on the cell surface can be identified by FTIR spectroscopy because each group has a unique energy absorption band (Jin and Bai, 2002). With this technique it was possible to study the carbohydrate variation of the CW under the influence of different additives.

The glucans content, and the β-glucan/ chitin ratio in particular, varied with the yeast strain and with the additive studied, suggesting that these species could potentially be sources of these polysaccharides. These considerations of the influence of growth parameters are important if the goal is to maximize CW yields, as would be the case in commercial situations. Furthermore, more studies are required on the chemical and physical properties of these polysaccharides to determine their structure and evaluate their industrial or medical applications. On the other hand, it is necessary to take into account the form of extraction of β-glucans and the different methods of determining the concentration in the CW.

8 | CONCLUSION

This study determined the influence of additives on yeast CW and biomass production. The 7 treatments were optimal for biomass production. The YPD medium (control) and the YPD with the 3 additives favored the percentage of CW for the *Saccharomyces* sp. 1 strain. For the *Saccharomyces* sp. 2 the optimal medium for the production of CW was YPD with SDS and ClNa, while for the *Candida* sp. strain it was the medium with EDTA and SDS.

The IR study of the 3 strains shows that the composition of CW contains chemical groups related to β-glucans. Future studies must be done to extract the β-glucans and determine their concentration in each CW.

The optimization of the production of CW and its components from yeast strains will allow its application in obtaining β-glucans for the food, pharmacological and cosmetic industries.

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