Antioxidant and prebiotic activity of (1→3)-β-D-glucan obtained from *Rhodotorula mucilaginosa*

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ABSTRACT
The β-Glucans has high biotechnological potential application in several industrial sectors. Then, the antioxidant and prebiotic activity of (1→3)-β-D-glucan obtained from the cell wall of R. Mucilaginosa yeast was evaluated. The strains tested Bifidobacterium Lactis and Bifidobacterium longum showed a comparable result with the commercially inulin. The digestibility of glucan was investigated in vitro by exposing them to artificial human gastric juice. The results show that not occurred hydrolysis of (1→3)-β-D-glucan under the studied conditions and the glucan remain undigested in 100%. Antioxidant capacity was evaluated by ABTS, DPPH and total antioxidant capacity test. The results of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity showed a maximum inhibition percentage of 51.00% and the test of total antioxidant capacity showed that 10 mg/mL of (1→3)-β-D-glucan shows the same result as the use of the 222.27 ± 4.3 µg/mL ascorbic acid. The obtained results show that the (1→3)-β-D-glucan extracted from the cell wall of R. mucilaginosa has the potential to be explored as a food ingredient with functional capacity.

Keywords: Polysaccharide, Glucan, Rhodotorula mucilaginosa, Antioxidant, Prebiotic.

1 INTRODUCTION

Glucans is a polysaccharide formed by subunits of D-glucose joined by glycoside bonds of type α or β, resulting in α-glucans and β-glucans, respectively (Hertzog, 2013). The β-Glucans receive special attention due to the high biotechnological potential they present, which has aroused the interest of several industrial sectors. The applications especially in the food, pharmaceutical and chemical sectors being used as: antitumor (Vannucci et al., 2013, anti-inflammatory (Valasques Junior et al., 2015), antimutagenic, antioxidant, hypoglycaemic and hypocholesteremic, thickened flocculants, stabilizers, emulsifiers and gelling agents (Banerjee, 2013, Oliveira Neto et al., 2016), anticoagulants, antithrombotic (El-Kashoury et al., 2016), antiviral (Soares, 2019), antioxidants (Nandi et al., 2014) and prebiotic (Zhao and Cheung, 2011).

The use in industrial scale of β-glucans of microbiological origin shows some advantages: production independence of environmental conditions; production in spaces relatively limited; facility of to *control the growth in the cultivation;* less susceptibility to the variation of its chemical and

physical properties; rapid production; existence of molecular genetics techniques that allow obtaining microbial polysaccharides with specific properties, which are not yet possible in vegetables (Luna, 2016).

Prebiotic activity has been widely researched due to its importance for health improvements of those who consume it. Prebiotics are defined as non-digested ingredients, that stimulating growth or metabolic activity of benefit bacteria present in the gastrointestinal microbiota (Gibson et al., 2010; Holscher). Recent studies with complex carbohydrates, such as polysaccharides, from various plant sources, shows that can be used as prebiotic. However, polysaccharides can also be synthesized by some microorganisms (Patel et al., 2010).

In addition to the prebiotic activity, the antioxidant activity of polysaccharides of microbiological origin has also been the subject of research and studies were carried out with this purpose (Sharma et al., 2010; Zhao et al., 2017; Lee et al., 2018).

Yeast and molds can synthesize β-glucans in the cell wall which serves to strengthen the cell structure and acts as a food reserve (Utama et al., 2021) Thus, the objective of this study was to evaluate the prebiotic and antioxidant activity of (1→3)-β-D-glucan extracted from cell wall of *R. Mucilaginosa* yeast isolated from semiarid region of Bahia.

2 MATERIAL AND METHODS

2.1 PRODUCTION AND EXTRACTION OF GLUCAN

The yeast *R. Mucilaginosa* it was obtained from the Collection of culture of Microorganimos of Bahia (CCMB, Feira de Santana, Brazil).

The strain of *R. mucilaginosa* was incubated in an MMS culture medium for activation of 4 days at 30ºC, the according with Valasques Junior et al, 2014. Flasks were inoculated with ten milliliters of cell suspension contend 10^9 UFC of *R. mucilaginosa*. After, 10 ml inoculum was inoculated in 90mL YM broth and incubated in orbital agitator (Tecnal, model 420) at 28°C for 120h at 100 rpm. After fermentation the medium was centrifuged at 9,000 rpm for 10 minutes and the cells were dried (55°C) until constant weight (Valasques Junior et al, 2014).

The powder of dry biomass (10 g) was extracted several times with 10 volumes of NaOH 0.1M. The extractions were done at 70ºC and 120 min. The suspension was centrifuged at 10.000×*g*for 10 min. It was added to the supernatant three times of the volume of absolute ethanol, stirred vigorously and left overnight at 4°C. The precipitated polysaccharides were centrifuged at 10.000×*g* for 30 min and the supernatant discarded. The precipitate containing the polysaccharides was dried at 55°C to a constant weight and the polymer was weighted by a scale (Sartorious ALC-110.4, Germany) (Valasques Junior al et., 2014).

2.1.1 Digestibility test of glucan

The evaluation of the prebiotic capacity of (1→3)-β-D-glucan was evaluated based on the ability to resist artificial gastric juice, enzymatic hydrolysis and promotion of the growth of probiotic bacteria.

2.1.2 Effect of hydrolysis of artificial human gastric juice

Polysaccharides were tested for acid resistance, compared to an inulin as a commercial prebiotic reference. The sample was dissolved in distilled water (0.1% w/v). Artificial human gastric juice was simulated using a hydrochloric acid containing (in g/L) buffer: sodium chloride (8); potassium chloride (0.2); di-sodium hydrogen phosphate dihydrate (8.25); sodium phosphate anhydrate (14.35); calcium chloride dihydrate (0.1); magnesium chloride hexahydrate (0.18), adjusted to pH 1 and pH 5 with the addition of 5 M (Korakli et al., 2002). The buffer was added to the sample solution (1% w/v) in the ratio 1:1 and incubated at 37°C for 6 hours. Samples (1 ml) were taken periodically at 0, 0.5, 1, 2, 4 and 6 hours. Inulin were used as controls. The content of reducing sugars in the samples was determined by the method of DNS (Miller, 1959) and total sugar was determined by the phenol-sulfuric acid method (Dubois et al., 1956). The percentage hydrolysis of the sample was calculated based on the reduction of the released sugar and the total sugar content of the sample (Korakli et al., 2002).

Hydrolysis% = (sugar content reducer released x 100)/ (Total sugar content-initial reducing sugar content). Where the reduction of the released sugar is the difference between the final and initial content.

2.2 EFFECT OF A-AMYLASE HYDROLYSIS IN GLUCAN

The enzymatic activity was determined according to the methodology used by Wichienchot et al., (2010) with modifications, using α-amylase (EC. 3.2.1.1) enzyme in solution containing 2 units/ml using sodium phosphate buffer (20 mM), sodium chloride (6.7 mM) at pH 4, 5, 6 and 7. The sample was prepared as a solution to 0.1% (w/v) in sodium phosphate buffer. 5 mL of enzyme solution were added to 5 mL of sample solution glucan. The reaction mixture was incubated in a water bath (37°C ± 1) for 6 h. The samples (1ml) were removed at 0, 0.5, 1, 2, 4 and 6 h to determine reducing sugar and total sugar. The percentage of hydrolysis was calculated based on the reduction of the released sugar and the total sugar content of the sample:

Hydrolysis% = (sugar content reducer released x 100)/ (Total sugar content-initial reducing sugar content).

2.2.1 Effect on the growth of probiotic bacteria

It was used MRS medium (Man, Rogosa & Shape) without the presence of the carbon source and with supplementation of 2.0% (1→3)-β-D-glucan or inulin (standard). Seven probiotic strains (*Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum, Bifidobacterium Brief, Bifidobacterium Bifidum, Bifidobacterium lactis and Bifidobacterium Longum* were tested to evaluate the ability to metabolize the glucan. Inoculums of the microorganisms were used in the percentage of 2% (v/v) of the active culture in the medium MRS supplemented with glucan. To inoculate the bacteria of the genus *Bifidobacterium* was used the Hungate technique (medium sterilized in flasks with rubber septum, and the inoculum was added with syringe through the septum). The cultures were incubated at 37°C for 48 hours in anaerobic conditions and samples were collected at times of 0, 12 and 24h for viable cell count, in medium MRS Agar.

2.3 ANTIOXIDANT ACTIVITY TESTS

2.3.1 Evaluation of total antioxidant capacity

Evaluation of total antioxidant capacity was performed according to the methodology described by Gonçalves (2014). An aliquot of 0.1 ml of each sample solution containing (1→3)-β-D-glucan at different concentrations (2, 4, 6, 8, and 10mg/mL^{-1} in distilled water) was mixed in a microtube with 1 ml of the reagent solution (0.6 M sulfuric acid, 28 mm sodium phosphate, 4 mm of molybdate ammonium chloride). The tubes were incubated at 95°C for 90 minutes. After cooling the samples at room temperature, the absorbance was measured at 695 nm. The results expressed from the equation of the straight obtained from the ascorbic acid calibration curve.

2.3.2 The determination of antioxidant activity by the ABTS • +

The determination of antioxidant activity by the ABTS • + method was performed according to the method described by Re *et al.,* (1999). The free radical ABTS was generated by the reaction of 5 mL of ABTS (7 mM) with 88 µL of potassium persulfate (2.45 µM,). The system was kept at room temperature (± 25ºC), during 16 hours in the absence of light. After ABTS $^{\bullet+}$ Radical was formed, dilution was done with distilled water until absorbance was obtained from 0.7000 ± 0.02 to 734 nm. The samples were prepared in distilled water and subsequently diluted at different concentrations (2, 4, 6, 8, 10mg/mL). The absorbance reading occurred with the reaction mixture containing 30 µL of sample and 3mL of ABTS solution, performed exactly after 6 minutes, from the radical mixture with the extract at a wavelength of 734 nm. The reaction control was prepared according to the procedure described above, without adding the sample, where the water is used to correct the baseline. The percentage of the decrease in absorbance was measured by the concentration and the ability to capture the ABTS $^{\bullet+}$ was calculated based on the decrease in the observed absorbance.

The inhibition percentage of the ABTS radical was calculated according to the formula:

% inhibition = ((Abs control – Abs sample)/Abs control) * 100.

2.4 THE ANTIOXIDANT ACTIVITY BY THE METHOD OF REMOVAL OF THE DPPH RADICAL

The antioxidant activity by the method of removal of the DPPH radical was assayed with two ml of sample mixed with 2 ml of DPPH 0.2 mM methanol solution and after 30 minutes of incubation, under light and at room temperature, the absorbance measurement of the sample was performed (Blois, 1958). The reaction control was prepared according to the procedure described above, without adding the sample. Methanol was used to correct the baseline. The percentage of the decrease in absorbance was measured by the concentration and the ability to capture free radicals and was calculated based on the decrease in the observed absorbance. The absorbance reading was performed at 517 nm. Ascorbic acid in the same concentrations of samples was used as standard control.

The decrease in the reading of the optical density of the samples was correlated with the control, establishing the percentage of discoloration of the DPPH radical. The percentage of DPPH radical discoloration was calculated according to the equation:

Inhibition% = ((Abs control – Abs sample)/Abs control) * 100

2.5 STATISTICAL ANALYSES

Data were statistically treated using Student's T test and Turkey test at 5.0% probability using the statistical package SISVAR 5.6.

3 RESULTS AND DISCUSSION

3.1 DETERMINATION OF PREBIOTIC ACTIVITY IN VITRO

The evaluation of the prebiotic capacity of the polysaccharide tested was evaluated considering the ability to resist gastric juice Digestibility of (1→3)-β-D-glucan, resistance to α amylase and promotion of bacterial growth of the genera *Lactobacillus* and *Bifidobacterium.*

The digestibility of (1→3)-β-D-glucan was evaluated to artificial human gastric juice and the action of α-amylase. The results show that not occurred hydrolysis of (1→3)-β-D-glucan under the studied conditions, for a period of 6 hours of observation. Glucan obtained from *R. mucilaginosa* shows high resistance to acids when compared to the reference prebiotic (inulin), which presented maximum hydrolysis of 19.5% at pH 2 after 6 hours of observation.

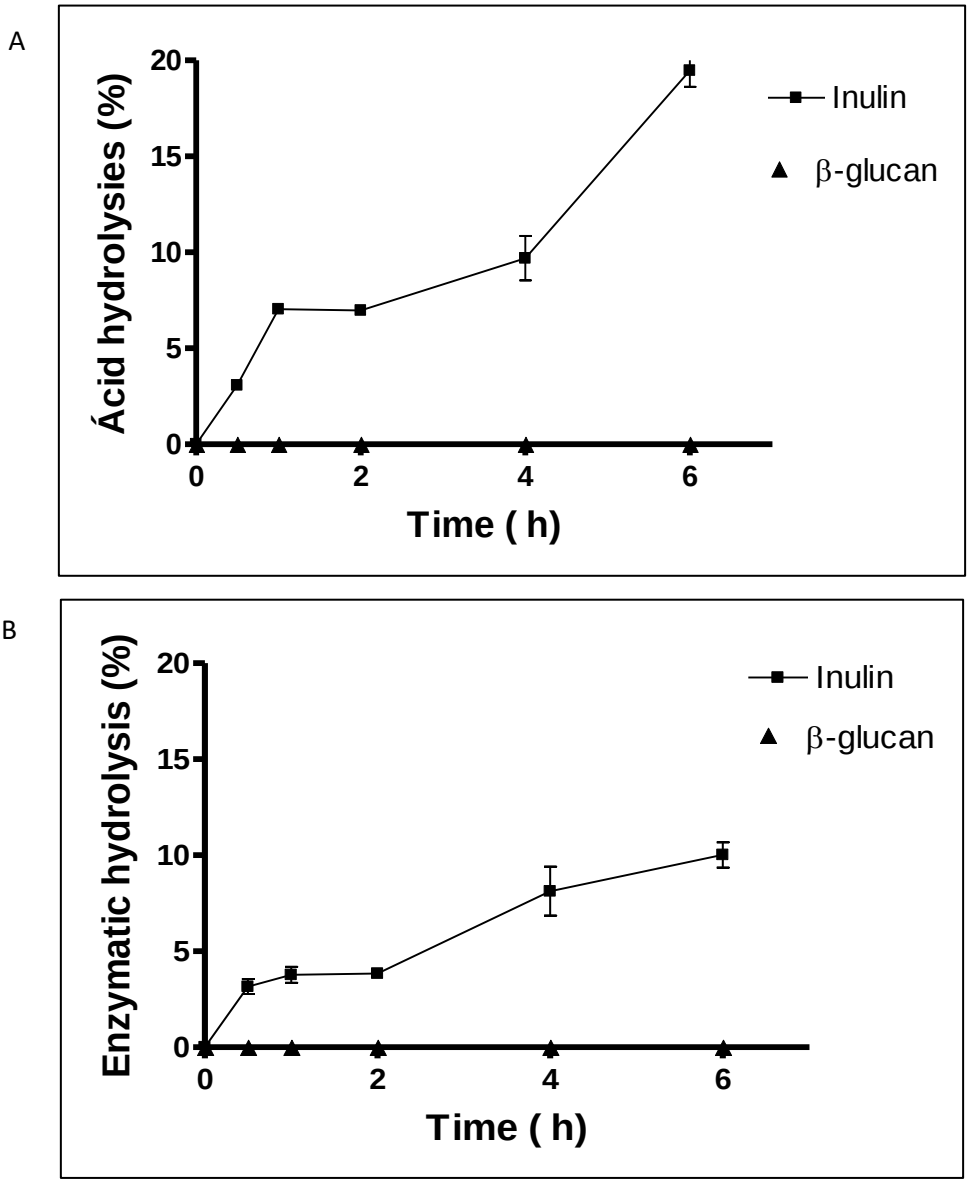
Similarly, (1→3)-β-D-glucan showed high resistance to the action of α-amylase and did not present reduction of reducing sugars in the presence of this enzyme. When compared to the reference prebiotic (inulin), it was possible to observe that (1→3)-β-D-glucan was more resistant than the

standard prebiotic, since inulin shows a hydrolysis percentage of 10.0% at pH 7 after 6 hours of observation (Figure 1).

The results suggest that (1→3)-β-D-glucan after ingested, may be able to pass through the gastrointestinal tract, without however, suffer the action of digestive juices with great possibility of ability to reach intact gastrointestinal tract.

Some carbohydrates, are not enzymatically degraded in the stomach or intestine and pass to the cecum and colon where they become 'colonic food' (Crittenden, 1999; Houdijk *et al.*, 1998; Kontula, 1999, Callaway et al., 2011).

Figure 1. Inulin and (1→3)-β-D-glucan digestibility. (A) Acid hydrolysis at pH 2 and (B) enzymatic hydrolysis at pH 7, incubated for 6 hours at 37 º C.



3.2 PROBIOTIC BACTERIA GROWTH STIMULATION

The ability of glucan to promote the growth of genera *Lactobacillus* and *Bifidobacterium* was determined in vitro and the results are presented in Table 1.

The initial inoculums were standardized in 7 ± 1 Log_{10} UFC/mL to facilitate the calculation of the actual growth of the microorganisms studied. The averages of the data obtained were compared by the T test (Table 1).

When comparing the growth stimulation of *L. casei, L. acidophilus, L. plantarum, B. bifidum* and *B. breve* strains, in medium supplemented with β glucan or inulin, it was observed that the strains developed the growth inferior to the control. The most satisfactory results were attributed to strain *B. Lactis* and *B. Longum.*

The strain *B. longum* after 12 hours of fermentation, showed a real growth of 2.5 log_{10} CFU/mL and 2.1 log_{10} CFU/mL, respectively, in medium supplemented with (1→3)-β-D-glucan and inulin, being superior to the group control. The Strain *B. Lactis* after 24 hours of fermentation showed no significant difference when compared to positive control, and the real growth of 2.0 Log_{10} CFU/mL, statistically equal to the positive control 2.1 Log_{10} CFU/ML. This shows the capacity of the microorganisms of *B. Lactis* and *B. Longum* to metabolize (1→3)-β-D-glucan, since their results may be compared to the prebiotic already widely recognized.

An explanation for the different behaviors observed in the studied strains may be correlated with the ability of the strains to produce enzymes as an example, glycoside hydrolases that are able to cleavage the glycoside bonds in the presence of water. It is a beneficial reaction of the probiotic microorganisms, since small molecules can be released by hydrolysis, which would facilitate the metabolization of the carbohydrate by the probiotic (Pokusaeva *et al*., 2011).

These data are in accordance with the study of Lambertus et al., (2008) that shows the bacteria of the genera *Lactobacilus sp* and *Bifidobacterium sp*. contains a high percentage of genes that present sequence coding for carbohydrate-modifying enzymes such as glycoside hydrolases, which in turn may have different substrate specificity.

Studies shows that *B. breve* and *B. longum* produce several carbohydrate-modifying enzymes among them the β-glucosidase belonging to the glycoside hydrolases family, being able to hydrolyze β-D-glucan and release glucose. This explains its ability to metabolize (1→3)-β-D-glucan since it is composed of monomers of D-glucose (Pokusaeva et al., 2011).

The results obtained in this study are in accordance with the results obtained by Zhao and Cheung, (2011), who evaluated the glucan from various origins (plant, bacteria, yeasts and algae) in growth of *B. Infantile strains, B. longum* , and *B. Adolescents*, observed in their results that the use of the tested carbohydrate, independent of the source and its differences in glycoside bonds shows an effect comparable to that of inulin.

Shia and collaborators (2018) when evaluating the prebiotic potential of polysaccharide (1 → 3)-β-D-glucan extracted from the bacterium *Alcaligenes faecalis* obtained as a result the real growth of *bifidobacterium sp* of 1.27 Log_{10} CFU/ml and *Lactobacillus sp* 1.05 log_{10} CFU/mL. The inulin showed a real growth of 1.01 and 0.41 log_{10} CFU/ML, respectively. For these authors, the tested polysaccharide can be potentially used as a food and functional ingredient.

Russo et al. (2012) tested the promoting growth of the bacteria *L. plantarum* WCFS1β-gal, *L. plantarum* WCFS1β-Gal and *L. acidophilus* in medium containing the exopolysaccharide β-D-glucan extracted from the microorganism *Pediococcus parvulus* and glucose, as positive control, and observed a growth variation of 1, 1 and 2 log cycles, respectively, showing a variation similar to that presented by the control group that presented a 1,1 and 2 log cycles. The results reported in this study revealed a positive effect of β-D-glucan on the growth of the investigated strains, suggesting that its use as prebiotic can positively modulate the growth of probiotic microorganisms.

The prebiotic effect, *in vitro,* of galactosaccharide produced by the fungus *scopulariopsis sp* for the strains *Lactobacillus acidophillus* and *Bifidobacterium animalis*, were tested results of the initial and final counts of microorganisms tested, which presented a difference of 3.88 log for *Lactobacillus acidophillus* and 4.60 log for *Bifidobacterium animalis (* Santos, 2010*).*

3.3 DETERMINATION OF ANTIOXIDANT ACTIVITY *IN VITRO*

The antioxidant capacity of glucan obtained from *R. mucilaginosa* was evaluated by three different methods: Total antioxidant capacity, DPPH scavenging activity and ABTS radical sequestration. The results of the antioxidant assays are expressed in Table 1.

Table 1 – Growth promoting of genera *Lactobacillus* and *Bifidobacterium* in MRS medium supplemented with 2.0% of (1→3)-β-D-glucan or inulin.

Microorganism	**(1→3)-β-D-glucan** (Log UFC/mL)	**Inulin** (Log UFC/mL)	**p**
L. casei	0.6±0.00	1.9±0.02	0.0000
*L. acidophilus**	0.7±0.00	2.0±0.06	0.0000
L plantarum	0.3±0.02	1.3±0.05	0.0000
*B. bifidum**	0.7±0.08	1.6±0.03	0.0000
B. short	0.6±0.05	1.9±0.1	0.0004
*B. lactis***	2.0±0.01	2.1±0.01	0.7129
*B. longum**	2.5±0.04	2.1±0.03	0.0012

Teste t-Student ($p<0,05$)
* Stationary Phase achieved after 12 hours of fermentation.
* * Stationary phase achieved after 24 hours of fermentation.
Average value (n = 3)

3.4 TOTAL ANTIOXIDANT CAPACITY

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex (Alam et al., 2012).

The evaluation of the total antioxidant capacity is based on the reduction of molybdenum^{6+} for molybdenum^{5+}, by antioxidant compound leading to the formation of the phosphomolybdenum complex, in acidic pH acquiring the greenish staining (RE *et al.,* (1999).

The value of the reduction power of the antioxidant samples under the molybdenum ion $^{6+}$ are represented in milligram equivalent ascorbic acid (mg/mL) per mg/ml sample. All samples had reduction power under the molybdenum ion^{6+}. Table 2 presents the results obtained expressed as ascorbic Acid equivalent (mg/mL).

Table 2: Total Antioxidant capacity of (1→3)-β-D-glucan the yeast Rhodotorula mucilaginosa.

1→3)-β-D-glucan (mg/mL)	*** Equivalence with ascorbic acid (mg/mL)**
	(1→3)-β-D-glucan
2	47.77-2.3^{d}
4	106.78±2.0^{c}
6	179.06±1.3^{b}
8	218.41 ± 3.1 ^{a}
10	222.27 ± 4.3 ^{a}
CV	3.97

Equal letters on the same line mean equal averages (P < 0.05)
Average value (n = 3)

The antioxidant activities of the tested samples evidenced a positive correlation between the concentrations analyzed and the resulting antioxidant activity. Thus, as the concentration of the samples increased, it showed a higher antioxidating activity, which was dependent on the dose. The results (Table 2) of the samples analyzed presents a correlation between the capacity presented by the tested polysaccharide and a commercial antioxidant (ascorbic acid). Thus, when tested at concentrations 2, 4, 6, 8 and 10 mg/mL of (1→3)-β-D-glucan and these presented the same antioxidant capacity as that presented by ascorbic acid 47.77 mg/ml, 106.78 µg/mL -179.06 µg/mL, 218.41 µg/mL and 222.27 µg/mL, respectively.

Arun and collaborators (2016) when testing the total antioxidant capacity of the exopolysaccharide extracted from *Halolactibacillus miurensis* when testing the concentration of 10 mg/mL sample found an equivalence of 2.7 units of ascorbic acid.

Meneses et al. (2016) when evaluating the total antioxidant capacity of the exopolysaccharide obtained from *gluconacetobacter diazotrophicus* used the maximum concentration of the sample of 75 mg/mL and managed to obtain a result Equivalent to 40 mg/mL ascorbic acid, values lower than those

found in our study where we obtained with the concentration of EPS of 10mg/mL the effect equivalent to 20.71 mg/mL ascorbic acid.

Similarly, Lima and collaborators, (2016) researching the total antioxidant capacity of polysaccharides, achieved an equivalence with 3 mg/ml of ascorbic acid, when he worked with 0.8mg/ml of galactomannan extracted from *Tylopilus ballouii.*

In this study the samples were able to reduce molybdenum$^{6+,}$ kidnapping free radicals formed *in vitro*, behaving in this way as an antioxidant. High equivalence with ascorbic acid compared to other literature reports show that the samples of polysaccharide tested show promising use as good antioxidants.

The sequestrating activity of the free radical DPPH has been widely used to determine the antioxidant effect of molecules. The technique is based on the capture of the DPPH radical by antioxidants, which when fixing an H^+ (removed from the antioxidant in study), loses color, becomes yellowish, leading to a decrease of absorbance at 515 nm, allowing to calculate, after the establishment of the equilibrium of Reaction, the amount of antioxidant in the sample equivalent to an antioxidant already known.

The analysis revealed that all tested samples had the ability to remove the DPPH radical**.** The results were expressed as a percentage of inhibition. The antioxidant activity of the samples was evaluated up to the concentration of 10 mg/mL (Table 3).

Table 3: Inhibition of DPPH Radical of polysaccharides obtained from R. mucilaginosa.

Sample (mg/mL	**(%) DPPH Radical Inhibition ***	
	(1→3)-β-D-glucan	**Acid Ascorbic**
2	29.67±1.3^{d}	100.0
4	36.82±1.5^{c}	100.0
6	39.32±1.1^{bc}	100.0
8	43.01±0.3^{b}	100.0
10	51.31 ± 0.7 ^{a}	100.0
CV	3.94	

Equal letters on the same line mean equal averages ($P < 0.05$)
Average Valor (n = 3)

The (1→3)-β-D-glucan presented the highest antioxidant activity, with a DPPH removal index of 51.92% achieved when the sample was tested at 10 mg/mL.

It is observed that the ability to remove the DPPH radical as presented with the test of total antioxidant capacity, is dose dependent, since the ability of removing the radical increases the measurement that increases the concentration of the sample. The results obtained were compared by the turkey test at 5.0% probability and the data were statistically significant ($P < 0.05$).

Abdhul et al (2014) when evaluating the antioxidant activity of exopolysaccharide obtained from strain *Enterococcus faecium*, obtained as a result percentages of DPPH radical inhibition similar to those found in this study. The obtained values ranged in approximately 20%, 40%, 50%, 55% and

65% for sample concentrations 2, 4, 6, 8 and 10mg/mL, respectively. These values are very close to those found in this study.

Our results agree with the results of the study conducted by Zhang et al. (2013) who evaluated the antioxidant capacity of exopolysaccharide isolated from *Lactobacillus plantarum* also achieved a percentage of inhibition of about 40% When you worked with the concentration of 4 mg/mL.

Liu et al, (2010), evaluated the antioxidant capacity of exopolysaccharide obtained from *Paenibacillus polymyxa* obtained about 40% of DPPH radical inhibition, value that is similar to those found in our study in the concentration of 4.0mg/mL.

Thus, it is observed that the ability to remove the DPPH radical was similar to the capacity found by the reference standards such as ascorbic acid that in the same concentrations, presented a percentage of inhibition of 100%.

To analyze the ability to remove the ABTS radical**,** all the analyzed samples showed few antioxidant activities, expressed as a percentage of inhibition of the ABTS radical. The antioxidant activity of the samples was evaluated up to the concentration of 10 mg/mL (Table 4).

Table 4: Inhibition of radical ABTS by (1→3)-β-D-glucan a sample produced by R. Mucilaginosa.

Sample (mg/mL)	**(%) ABTS Radical Inhibition**	
	(1→3)-β-D-glucan	**Ac. Ascorbic**
2	5.08±0.3[a]	98.68
4	5.65±0.8[a]	98.68
6	5.38±0.2[a]	98.70
8	5.00±0.1[a]	98.58
10	5.10±0.6[a]	98.64

Equal letters on the same line mean equal averages ($P < 0.05$)
Average value (n = 3)

This method is based on the generation of the ABTS radical, which presents a greenish blue color, through the reaction of the ABTS with potassium persulfate. The addition of an antioxidant results in the reduction of the ABTS radical, promoting the loss of the color of the reactional medium, which allows calculating the percentage of inhibition of the radical ABTS (RE, 1999).

Yang et al., (2014) to evaluate the antioxidant capacity of the extracted polysaccharide from *Panax japonicus* obtained a perceptual inhibition of the abts radical of about 80%. Similarly, when evaluating the antioxidant capacity of polysaccharide from *flammulin velutipes*, it was similar to that of ascorbic acid when the same concentrations were used.

Antioxidants can act by different mechanisms of action such as: the sequestration of free radicals, complexing with metal ions, decomposition of peroxide, and inhibition of enzymes responsible for the generation of Free radical (Vasconcelos et al., 2006).

The results showed that (1→3)-β-D-glucan obtained through *R. Mucilaginosa* yeast has a remarkable effect on the elimination of free radicals attested by DPPH radical removal techniques and total antioxidant capacity, on the other hand, it has little efficiency in the decrease of the ABTS Radical.

Nandi and collaborators (2014) researching the antioxidant potential of (1→3)-β-D-glucan extracted from the fungus *Russula albonigra*, using the radical hydroxyl tests, superoxide radical, ferric ion reduction power and β-carotene assay, obtained as a result a good percentage of antioxidant activity for the tests methods.

Thus, it is perceived that polysaccharides extracted from the *R. Mucilaginosa* yeast showed to be promising in the antioxidant use, revealing a possible bioactive substance of great interest to several industrial sectors.

4 CONCLUSION

The tested bacteria belonging to the genera *Lactobacillus* and *Bifidubacterium* showed the ability to metabolize the (1→3)-β-D-glucan, and satisfactory growth comparable with inulin control was found in the species *B. Lactis* and *B. Longum.* After 24 hours of fermentation showed a growth 2.0 $\log_{10}$UFC/ml statistically comparable with the control 2.1 $\log_{10}$ CFU/mL. The species *B. Longum* after 12 hours of fermentation showed a real growth higher than the control group 2.5 and 2.1 Log $_{10}$CFU/mL, respectively.

In the test of total antioxidant capacity, the samples analyzed (10 mg/mL) can be equated to 222.07 M g/ml with ascorbic acid. The DPPH radical inhibition test showed that (1→3)-β-D-glucan may act in the sequestration of the DPPH radical and the maximum inhibition percentage of 51.00% was obtained with 10mg/mL of (1→3)-β-D-glucan.

Therefore, the (1→3)-β-D-glucan polysaccharide produced by the yeast *R. Mucilaginosa* exhibited antioxidant and prebiotic action, showing potential to be used as an ingredient in functional foods.

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