I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ** XV SHEB ** XV ENZITEC FLORIANÓPOLIS ** 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
INDUSTRIAL MICROBIOLOGY: PROSPECTING AND APPLIED MOLECULAR BIOLOGY

ANALYSIS OF MAXIMUM SPECIFIC GROWTH RATES OF YEASTS ISOLATED FROM DECAYING ORANGES

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ABSTRACT

Brazilian orange production is mainly destined for processing by the juice industry. However, half of the mass of processed fruits is estimated to result in waste, including peels, pulp, and seeds. This raw material is mainly composed of cellulose, hemicellulose, and pectin, which can be converted into bioproducts through the fermentative metabolism of microorganisms such as yeasts. To achieve this, researchers are exploring the bioprospecting of yeasts capable of metabolizing the carbohydrates present in these residues. In this context, the present study isolated 38 yeast strains from decaying oranges, evaluating their growth capacity on different carbohydrates and estimating the maximum specific growth rates (μmax). Overall, the strains showed higher μmax values when grown on glucose, fructose and galactose, but some also stood out in media containing xylose and galacturonic acid. Five strains showed high growth capacity on galacturonic acid (CHAP-048, CHAP-054, CHAP-065, CHAP-069, and CHAP-070), with μmax values that varied between 0,17 h^{-1} and 0,20 h^{-1}. Therefore, the isolated strains appear promising for use in future studies to discover their capabilities for consuming and fermenting carbohydrates derived from orange waste, especially xylose and galacturonic acid.

Keywords: Pectin. Galacturonic acid. Orange peel. Yeasts. Bioprospecting.

1 INTRODUCTION

The orange stands as one of the Brazilian primary commodities. In 2022, the country produced 16.9 million tons of this citrus fruit.[1] Most of this production is destined for processing for the juice industry, with Brazil dominating 67% of global orange juice production.[2] However, it is known that about 50% of the mass of processed fruits consists of waste such as peels, pulps, and seeds.[3,4] These byproducts could be used in biotechnological processes to create new high-value products, benefiting sectors such as pharmaceuticals, food, and biofuels.[5] This plant residue is composed, on average, of 18.1% cellulose, 13.1% hemicellulose, and 28.5% pectin, known as insoluble polysaccharides.[6] Cellulose is a polysaccharide formed exclusively by glucose molecules, while hemicellulose is a heteropolymer mainly represented by xylan, a xylose polysaccharide. As for pectin, the major polymer in this plant residue, it is formed by a linear chain of galacturonic acid molecules linked together through β-1,4 glycosidic bonds; these polymers branch out into other chains formed by carbohydrates such as galactose, rhamnose, arabinose, and xylose.[7,8] In addition to polysaccharides, fruit peels may also contain soluble mono and disaccharides such as glucose, fructose, and sucrose.[9]

Since orange residues are formed by carbohydrate polymers and soluble sugars, these saccharides can be transformed into bioproducts through the fermentative metabolism of microorganisms such as yeasts. These yeasts, found in various natural environments, can catalyze the fermentative processes that convert sugars into products, such as bioactive compounds, beverages, and biofuels.[10,11] In this context, the present study aimed to isolate yeasts from decomposing orange peels, aiming to discover new strains capable of metabolizing the carbohydrates present there. Furthermore, using the isolated yeasts, cellular growth analyses were carried out on microscale in media containing different sugars as carbon sources, in order to screen for the best growth rates and, thus, the highest biotechnological potential.

2 MATERIAL & METHODS

The yeasts were isolated from decaying oranges harvested on rural properties in the cities of Chapecó and Xaxim (Santa Catarina, Brazil), by adapting the previously described protocol.[12] For this, 1,0 g was randomly taken from the oranges and inoculated into a liquid medium composed of 6,7 g L^{-1} of yeast nitrogen base and 10 g L^{-1} of xylose. After cellular growth, a portion of cells from the liquid medium was plated on Petri dishes with the same media, from which yeast colonies were isolated.

For cellular growth on microscale, the yeast cells were pre-grown in rich media (20 g L^{-1} of glucose, 20 g L^{-1} of peptone, and 10 g L^{-1} of yeast extract) for 16 hours. Aliquots of these precultures were taken and used to inoculate (Optical Density ~0,1 at 570 nm) synthetic media containing 6,7 g L^{-1} of yeast nitrogen base without amino acids, supplemented with 20 g L^{-1} of glucose, xylose, fructose, galactose, or galacturonic acid. The yeasts were cultivated in 96-well plates with 100 µL of culture medium in each well and sealed with Sealing Film (E & K Scientific). The plates were incubated at 30°C with orbital agitation (amplitude 2 mm) in a multifunctional reader (TECAN ECHISTO INFINITE M200 PRO), where cellular growth was monitored by Optical Density at 570 nm. The maximum specific growth rate [μmax (h^{-1})] was determined by the geometric method of derivative calculation following the protocol by Schmidell et al. (2001).[13]

3 RESULTS & DISCUSSION

The first part of this work consisted of isolating yeasts from oranges in decomposition. As a result, 38 strains were isolated, and each isolated strain was identified with the acronym CHAP and a number that varied between 45 and 82 (Table 1). The strains were evaluated for cell growth kinetics on microscale in media containing some of the carbohydrates found in orange peels (glucose, xylose, fructose, galactose, and galacturonic acid) and the maximum specific growth rate (μmax) was calculated for each of the yeasts on each of the carbohydrates tested. This parameter is interesting for optimizing fermentation processes, adjusting cultivation conditions and maximizing the production of desirable compounds.[14,15]

As can be seen in Table 1, which summarizes all calculated μmax values, the strains mostly presented the highest maximum specific growth rates when grown in glucose, fructose, and galactose. Strain CHAP-045 showed the highest μmax in fructose and galactose (0,56 h^{-1} and 0,53 h^{-1}, respectively), while strain CHAP-048 showed the highest μmax in glucose (0,59 h^{-1}). In fact, many yeasts have been characterized by their ability to metabolize mainly sugars such as glucose and fructose, found in ripe fruits.[16] Furthermore, galactose has been identified as the pectic sugar most fermented by yeast.[17] On the other hand, for ten strains (CHAP-052, CHAP-053, CHAP-054, CHAP-055, CHAP-057, CHAP-058, CHAP-060, CHAP-065, CHAP-066 and CHAP-064), the μmax values calculated from the growth curves in media containing xylose as a carbon source (μmax values that varied between 0,25 h^{-1} and 0,31 h^{-1}) were very close to or even higher than those found from the curves in glucose, fructose and/or galactose (Table 1). These yeasts may be interesting to be tested in lignocellulosic hydrolysates from biomasses such as bagasse and sugarcane straw, for example, which have high concentrations of xylose, aiming to test the production of second-generation ethanol.[18]

Table 1: Maximum specific growth rate [μmax (h^{-1})] calculated for each strain based on experimental cell growth data obtained during microscale cultures. Data represent the mean and standard deviation of two independent experiments. For some strains highlighted in the text, the values are in bold.

Strain	Carbohydrate				
	Glucose	Xylose	Fructose	Galactose	Galacturonic Acid
CHAP-045	0,34 ± 0,00	0,27 ± 0,02	**0,56 ± 0,06**	**0,53 ± 0,01**	-
CHAP-046	0,38 ± 0,06	0,20 ± 0,00	0,37 ± 0,04	0,36 ± 0,06	-
CHAP-047	0,36 ± 0,02	0,25 ± 0,00	0,40 ± 0,02	0,28 ± 0,01	-
CHAP-048	**0,59 ± 0,06**	0,29 ± 0,04	0,27 ± 0,01	0,07 ± 0,00	**0,18 ± 0.02**
CHAP-049	0,37 ± 0,00	0,24 ± 0,04	0,39 ± 0,00	0,43 ± 0,00	0,13 ± 0,00
CHAP-050	0,42 ± 0,02	0,22 ± 0,01	0,37 ± 0,01	0,36 ± 0,02	0,12 ± 0,00
CHAP-051	0,45 ± 0,03	0,25 ± 0,01	0,42 ± 0,03	0,41 ± 0,02	-
CHAP-052	0,36 ± 0,02	**0,30 ± 0,01**	0,32 ± 0,02	0,36 ± 0,02	0,14 ± 0,01
CHAP-053	0,35 ± 0,05	**0,31 ± 0.02**	0,37 ± 0,05	0,32 ± 0,02	-
CHAP-054	0,25 ± 0,02	**0,28 ± 0,03**	0,28 ± 0,02	0,30 ± 0,04	**0,19 ± 0,01**
CHAP-055	0,36 ± 0,02	**0,30 ± 0,02**	0,34 ± 0,02	0,30 ± 0,03	-
CHAP-056	0,45 ± 0,06	0,31 ± 0,05	0,41 ± 0,03	0,30 ± 0,04	-
CHAP-057	0,31 ± 0,02	**0,30 ± 0,01**	0,32 ± 0,02	0,31 ± 0,02	-
CHAP-058	0,30 ± 0,01	**0,31 ± 0,03**	0,29 ± 0,02	0,33 ± 0,02	0,10 ± 0,00
CHAP-059	0,41 ± 0,03	0,26 ± 0,02	0,40 ± 0,03	0,38 ± 0,01	-
CHAP-060	0,33 ± 0,03	**0,30 ± 0,01**	0,38 ± 0,04	0,35 ± 0,02	-
CHAP-061	0,34 ± 0,02	0,23 ± 0,02	0,28 ± 0,03	0,27 ± 0,02	-
CHAP-062	0,33 ± 0,01	0,22 ± 0,02	0,35 ± 0,01	0,27 ± 0,01	-
CHAP-063	0,41 ± 0,02	0,28 ± 0,02	0,43 ± 0,03	0,44 ± 0,02	-
CHAP-064	0,37 ± 0,04	0,26 ± 0,01	0,45 ± 0,03	0,49 ± 0,00	-
CHAP-065	0,29 ± 0,02	**0,30 ± 0,02**	0,39 ± 0,03	0,28 ± 0,01	**0,17 ± 0,02**
CHAP-066	0,28 ± 0,02	**0,27 ± 0,02**	0,30 ± 0,02	0,36 ± 0,03	-
CHAP-067	0,37 ± 0,01	0,25 ± 0,03	0,38 ± 0,03	0,35 ± 0,01	-
CHAP-068	0,40 ± 0,01	0,20 ± 0,01	0,25 ± 0,01	0,45 ± 0,02	-
CHAP-069	0,28 ± 0,04	0,24 ± 0,03	0,49 ± 0,02	0,51 ± 0,06	**0,20 ± 0,02**
CHAP-070	0,28 ± 0,02	0,20 ± 0,00	0,31 ± 0,03	0,35 ± 0,04	**0,19 ± 0,02**
CHAP-071	0,25 ± 0,01	0,20 ± 0,01	0,29 ± 0,02	0,39 ± 0,06	-
CHAP-072	0,26 ± 0,02	0,19 ± 0,02	0,28 ± 0,02	0,25 ± 0,01	0,09 ± 0,02
CHAP-073	0,33 ± 0,02	0,20 ± 0,00	0,25 ± 0,02	0,28 ± 0,03	0,08 ± 0,02
CHAP-074	0,26 ± 0,04	**0,25 ± 0,02**	0,29 ± 0,03	0,30 ± 0,02	0,13 ± 0,01
CHAP-075	0,25 ± 0,02	0,22 ± 0,03	0,27 ± 0,04	0,35 ± 0,04	0,14 ± 0,02
CHAP-076	0,34 ± 0,01	0,19 ± 0,01	0,30 ± 0,02	0,35 ± 0,01	-
CHAP-077	0,35 ± 0,02	0,22 ± 0,02	0.38 ± 0,01	0,39 ± 0,03	-
CHAP-078	0,39 ± 0,03	0,18 ± 0,02	0,28 ± 0,02	0,40 ± 0,03	-
CHAP-079	0,40 ± 0,04	0,27 ± 0,02	0,29 ± 0,02	0,41 ± 0,03	-
CHAP-080	0,39 ± 0,02	0,22 ± 0,03	0,42 ± 0,01	0,45 ± 0,04	-
CHAP-081	0,32 ± 0,03	0,29 ± 0,02	0,31 ± 0,02	0,40 ± 0,02	-
CHAP-082	0,25 ± 0,02	0,12 ± 0,02	0,29 ± 0,02	0,35 ± 0,02	-

(-) strain did not show cell growth on carbohydrate.

Interestingly, thirteen strains (CHAP-048, CHAP-049, CHAP-050, CHAP-052, CHAP-054, CHAP-058, CHAP-065, CHAP-069, CHAP-070, CHAP-072, CHAP-073, CHAP-074, and CHAP-075) demonstrated cell growth in media containing galacturonic acid as a carbon source. Since this is the primary pectic sugar[19], the cell growth curves in galacturonic acid were illustrated in Figure 1. Five of these strains stood out (CHAP-048, CHAP-054, CHAP-065, CHAP-069 and CHAP-070), exhibiting the highest μmax, which varied from 0,17 h^{-1} to 0,20 h^{-1} (Table 1). It is worth noting that galacturonic acid is not innately metabolized by yeasts

relevant to the biotechnology industry, such as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Yarrowia lipolytica* and *Pichia pastoris*.[5] In this sense, the discovery of new yeasts with the capacity to metabolize this carbon source is desirable for the biotechnology industry that uses orange waste.[17] Therefore, the thirteen strains isolated in the present study, which exhibited cellular growth from this carbohydrate, could be further investigated to understand their galacturonic acid consumption profiles or even their genetic characteristics related to the metabolism of this monosaccharide in yeast.

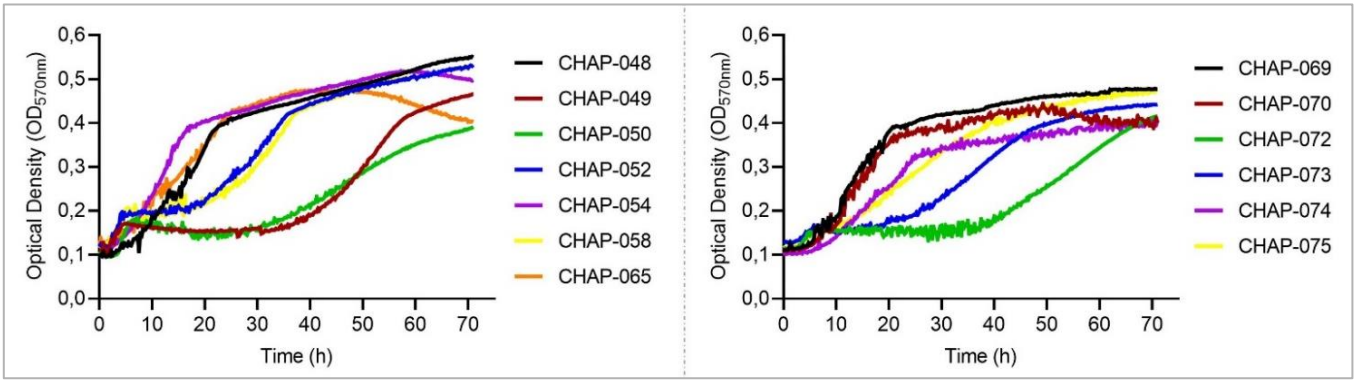

Figure 1: Microscale growth curves in media containing galacturonic acid as a carbon source for thirteen strains, of the 38 analyzed, that showed growth in this carbohydrate. Data represent the mean of two independent experiments.

4 CONCLUSION

In the present work, 38 yeast strains were isolated from decaying oranges and evaluated through their ability to grow in media with the carbohydrates glucose, xylose, fructose, galactose, and galacturonic acid. The results demonstrate that, overall, the strains exhibited the highest maximum specific growth rate (μmax) values when grown in glucose, fructose, and galactose. However, for ten strains, the μmax values calculated from growth curves in media containing xylose were very close to or even higher than those found from curves in glucose, fructose, and/or galactose. Additionally, thirteen strains showed the ability to grow in galacturonic acid, with five of them (CHAP-048, CHAP-054, CHAP-065, CHAP-069, and CHAP-070) showing the highest μmax values from curves in this carbon source. Thus, our isolates can be further studied with the aim of being employed in biorefinery environments with lignocellulose- and pectin-rich substrates.

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ACKNOWLEDGEMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq, 406564/2022-1; 308830/2023-7; 309047/2023-4; 150719/2023-0), and Research and Innovation Support Foundation of Santa Catarina State (FAPESC, 2023TR000234).