ANALYSIS OF CELL GROWTH, SUGAR CONSUMPTION, AND SECONDARY METABOLITES PRODUCTION BY FLOWERS- AND INSECTS-ASSOCIATED YEASTS

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ABSTRACT

The relationship between flowers, pollinating insects, and yeast is of great ecological importance. Yeasts ferment the sugars in floral nectaries, producing secondary metabolites that volatilize to a greater or lesser extent and attract insects to the flowers. Then, pollination occurs, insects feed, and yeasts can access these animals' bodies to inhabit them outside the flowering seasons. However, due to the commercial importance of the compounds produced by yeast metabolism, this relationship has also become of great biotechnological interest. Therefore, the present study sought to isolate yeasts from flowers and insects to evaluate them in agitated-flask cultures with sugars found in plants or in these invertebrates' guts. Three yeast strains were isolated and identified: CHAP-222 (*Aureobasidium* sp.), CHAP-223 (*Papiliotrema rajasthanensis*) and CHAP-242 (*Meyerozyma caribbica*). The three yeasts exhibited growth in the five available carbohydrate sources, with CHAP-242 showing the fastest exponential growth. Among the secondary metabolites analyzed, 2-Phenylethanol was the most prevalent in the samples, having been produced by all strains in practically all conditions evaluated. Thus, the yeasts analyzed demonstrate potential for producing compounds with high added value from simple substrates.

Keywords: 2-Phenylethanol. Eucalyptol. Fructose. Tryptophol. Xylose.

1 INTRODUCTION

The attraction that plants exert on insects may come from chemical signals released by floral nectaries-dwelling yeasts[1]. Given the high concentration of sugars in flower nectar (especially sucrose, glucose, and fructose), these microorganisms find nectaries a favorable environment for their growth[2]. However, in seasons without flowering, these yeasts seek the gastrointestinal tract of insects as a habitat for prolonged periods, aiming for safety, reproduction, and transport[3]. Interestingly, in the intestines of these animals, yeasts can find other carbohydrates, such as xylose and cellobiose[4].

The chemical signals mentioned above are secondary products of yeast metabolism (secondary metabolites), normally alcohols, organic acids, and terpenoids, with a greater or lesser degree of volatility. In addition to their ecological roles, these compounds have great biotechnological relevance due to their various applications in different industry sectors[1]. In this context, the present work sought to evaluate the growth of yeasts isolated from plants and insects when exposed to different carbon sources (Sucrose, Cellobiose, Glucose, Xylose, and Fructose) and to analyze the production of secondary metabolites.

2 MATERIAL & METHODS

The yeast strains CHAP-222, CHAP-223, and CHAP-242 were isolated from Fedegoso flowers (*Senna macranthera*) and macerates of the beetle *Astylus variegatus* and the bee *Scaptotrigona postica*, respectively. In order to isolate the strain from the flower, the floral nectar was scraped with a sterile swab, followed by streaking in YNB solid medium (6.7 g/L of yeast nitrogen base) with 10 g/L of xylose, 0.2 g/L of chloramphenicol and 20 g/L of agar. For the isolation from insects, bees and beetles were collected, macerated, and inoculated in YNB liquid medium also containing 10 g/L of xylose and 0.2 g/L of chloramphenicol. After 3–5 days of cultivation at 30 ºC, 10 µL loops were removed from the medium and streaked on solid media with the same composition (added with 20 g/L of agar). Pure cultures were obtained from selecting isolated colonies with typical yeast morphology. The strains were then taxonomically identified by analyzing the internal transcribed spacer (ITS) as we previously described[5]. The amplified DNA was concentrated, cleaned and sequenced in an ABI 3130 Genetic Analyzer automated sequencing system (Life Technologies, California, USA) using BigDye v3.1 and POP7 polymer. The sequences were assembled, edited, and aligned using the program MEGA6. Finally, the sequences obtained were compared with those included in the GenBank (https://www.ncbi.nlm.nih.gov) using its Basic Local Alignment Search Tool (BLAST).

The three strains had their cell growth evaluated in YP media (10 g/L of yeast extract and 20 g/L of peptone, pH 5.0) containing, alternately, 20 g/L of xylose, fructose, glucose, and cellobiose, or 150 g/L of sucrose. Cultures were carried out at 30°C and 145 rpm for 48 h. For cell growth curves, samples of the medium were harvested three times a day, and their optical densities (OD) were analyzed in a spectrophotometer at 570 nm. Aliquots were taken for analysis of sugar consumption through high-performance liquid chromatography (HPLC) with the AMINEX HPX-87P column (Bio-Rad). At the end of the cultures, all media were transferred to Falcon tubes that underwent centrifugation at 8500 g for 5 min, and the supernatants were reserved. With a separation funnel, 20 mL of each supernatant was mixed with 6.67 mL of dichloromethane for liquid-liquid extraction, following an

adaptation of the protocol described by Roque and coworkers[6]. Next, the nonpolar phase containing dichloromethane was sent to a gas chromatograph coupled to a mass spectrometer (GC-MS) to identify the secondary metabolites produced by the evaluated yeasts. As a negative control, culture media before the inoculum also underwent the same extraction process and chromatographic analysis. The library used for mass spectrometry was NIST08s.

3 RESULTS & DISCUSSION

Based on ITS sequencing, the strains CHAP-222, CHAP-223, and CHAP-242 were taxonomically identified, respectively, as *Aureobasidium* sp., *Papiliotrema rajasthanensis*, and *Meyerozyma caribbica*. All strains were able to grow using the five carbohydrates tested as carbon sources (Figure 1). However, it was possible to observe up to two times greater growth in the bee-isolated yeast CHAP-242. Furthermore, this strain showed a consumption of 100% of all sugars, with the exception of cellobiose. The three strains produced five secondary metabolites with diverse biotechnological applications (Table 1). This analysis considered compounds with a Similarity Index (SI) ≥ 90% calculated by the NIST08s mass spectrometer library.

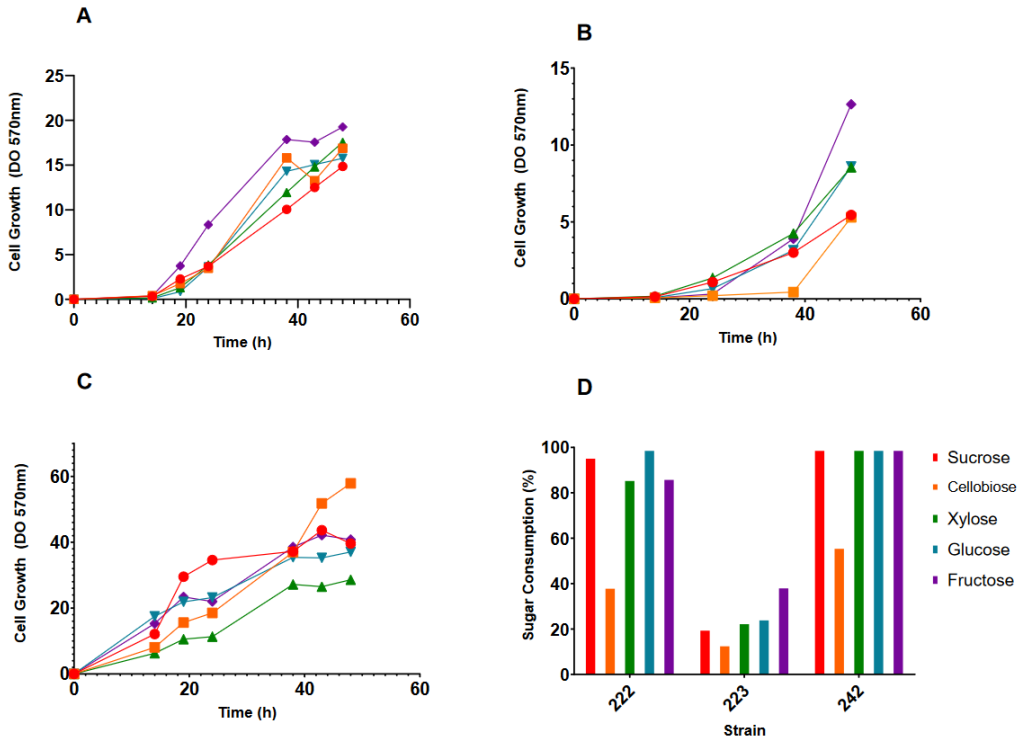

Figure 1. Cell growth of the tested strains, Aureobasidium sp. CHAP-222 (A), Papiliotrema rajasthanensis CHAP-223 (B) and Meyerozyma caribbica CHAP-242 (C) when cultivated in media with xylose (green), fructose (purple), cellobiose (orange), glucose (blue) and sucrose (red). Sugar consumption by the tested strains (D).

Table 1. Secondary metabolites produced by the yeasts and their industrial applications.

STRAIN	Compound	Application
222, 223, 242	2-Phenylethanol	Home care, flavoring agent, pesticide, personal cares, Preservative.
223	Eucalyptol	Pharmaceuticals, flavoring agent, fragrance, disinfectant and solvent;
223	Oleic Acid	Used in industry cosmetic, Pharmaceutical, food and Plastificants
223	Stearic Acid	Used in industry cosmetic, Pharmaceutical, food and textile
242	Tryptophol	Yeast metabolite, organic synthesis of tryptamines and the drug indomine.

*Applications according to PubChem. Available in: https://pubchem.ncbi.nlm.nih.gov/

Figure 2 indicates an expressive production of 2-Phenylethanol by the three strains tested. It was present in the cultures carried out in the five available carbon sources, with peak areas greater than the other compounds analyzed. CHAP-242 showed production of this compound while cultivated in the five sugars, especially on Sucrose, with a peak area of 46.9%. GC/MS peak area data represent the relative abundance of each compound, according to what has been described elsewhere[7]. Interestingly, this compound is one of the main responsible for attracting pollinators in angiosperms[8].

2-Phenylethanol is a superior alcohol obtained by yeasts through phenylpyruvate, a metabolite used in the biosynthesis route of the amino acid phenylalanine. Phenylpyruvate is decarboxylated and reduced to form 2-Phenylethanol via the Ehrlich pathway[9]. Furthermore, some yeasts synthesize 2-Phenylethanol again via the Shikimate or Cinnamate pathway[10]. Therefore, yeasts are capable of producing this compound through the catabolism of sugars and/or the anabolism of L-phenylalanine.

Other compounds were also detected, but with lower prevalence. Tryptophol, produced by CHAP-242 in a medium containing glucose, is an important compound that can also be converted by yeast into Indole-3-acetic acid (IAA). Indole-3-acetic acid is a plant auxin that aids in plant development and growth[11]. Eucalyptol was observed in CHAP-223, with a peak area of 19.94% in xylose. This molecule is an important terpene with bioactive properties, and it is widely used in the pharmaceutical industry. Terpenes are produced by yeast through the oxidation of pyruvate, generating Acetyl-CoA, which in turn is destined for the mevalonate pathway[12]. Fatty acids such as Steric and Oleic Acid were also obtained in small quantities by CHAP 223 (Figure 2).

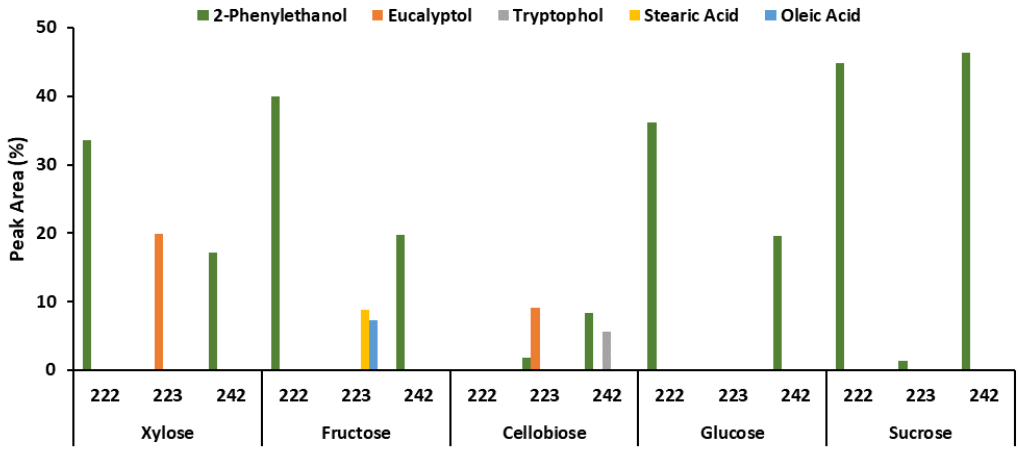

Figure 2. Relative abundance of secondary metabolites (2-Phenylethanol, Eucalyptol, Tryptophol, Stearic Acid, Oleic Acid) produced by the yeasts *Aureobasidium* sp. CHAP-222, *Papiliotrema rajasthanensis* CHAP-223 and *Meyerozyma caribbica* CHAP-242 when cultivated in media with xylose, fructose, cellobiose, glucose or sucrose as carbon sources.

4 CONCLUSION

Our results highlight the biotechnological potential of yeasts associated with plants and insects. The microorganisms analyzed demonstrated, through their metabolism, the ability to generate several compounds with high added value. In this context, 2-Phenylethanol stood out, with peak areas twice as large as those observed for other compounds. Furthermore, all strains showed the ability to grow in media with the five sugars tested, thus demonstrating that these yeasts can transform low-cost substrates, such as lignocellulosic plant residues.

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