Isolation and Characterization of Yeasts Able to Assimilate Sugarcane Bagasse Hemicellulosic Hydrolysate and Produce Xylitol Associated with Veturius transversus (Passalidae, Coleoptera, and Insecta)

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Yeasts are an important component of insect gut microbial content, playing roles such as degradation of polymers and toxic compounds, biological control, and hormone, vitamin, and digestive enzyme production. The xylophagous beetle gut is a hyperdiverse habitat and a potential source of new species with industrial abilities such as enzyme production, pentose fermentation, and biodetoxification. In this work, samples of Veturius transversus (Passalidae, Coleoptera, and Insecta) were collected from the Central Amazon Rainforest. Their guts were dissected and a total of 20 microbial colonies were isolated using sugarcane bagasse hemicellulosic hydrolysate. They were identified as having 10 distinct biochemical profiles, and genetic analysis allowed identification as three clades in the genera Candida, Williopsis, and Geotrichum. All colonies were able to assimilate D-xylose and 18 were able to produce xylitol, especially a strain of Geotrichum, with a maximum yield of 0.502 g g⁻¹. These results agree with a previous prediction that the microbial community associated with xylophagous insects is a promising source of species of biotechnological interest.

1. Introduction

Yeasts are microorganisms of the Fungi kingdom, distributed in the phyla Ascomycota, Basidiomycota, and Deuteromycota [1]. On the other hand, beetles are the most abundant order of insects (Coleoptera, Insecta, Arthropoda, and Metazoa), with more than 400,000 species currently described [2].

The association of yeasts with beetles was decisive in the evolutionary success of these insects, the microbiota being indispensable to them, playing fundamental roles such as in the synthesis of amino acids, lipids, pheromones, and digestive enzymes and in biodetoxification [3, 4]. According to Suh et al. [5], the microbial content of a xylophagous beetle’s gut is a hyperdiverse source of undescribed species.

Xylitol is a promising polyol of five carbons, with medical applications in middle ear otitis [6] and obesity prevention [7]. It is obtained from D-xylose reduction, performed by microbial fermentation or a chemical process [8]. As this latter releases a high number of by-products, demanding several steps for purification, sampling efforts aiming to isolate microbes with ability to produce xylitol keep being necessary.

Recent yeast and yeast-like fungi sampling efforts have increased the number of known species and strains able to produce vitamins, enzymes, and other products from fermentation of sugars such as ethanol and xylitol [9]. In this way, isolation and characterization of wild-type yeasts and yeast-like fungi remains an important approach.

The aim of this work was to isolate and characterize yeasts associated with the xylophagous beetle Veturius transversus (Passalidae, Coleoptera, and Insecta) able to assimilate sugarcane bagasse hemicellulosic hydrolysate (SBHH) as sole carbon source and produce xylitol by D-xylose fermentation.
2. Material and Methods

Under authorization (protocol number 34652-1) of the Instituto Chico Mendes de Conservação da Biodiversidade (Brazilian authority for biodiversity access), 15 beetles were collected from the Central Amazon Forest (3° 06’ 05.20” S, 59° 58’ 23.14” W). They were identified as *V. transversus*, a highly representative passalid beetle in this region. Three samples were deposited in the Entomological collection Paulo Burnheim (UFAM, Brazil).

The beetles were washed in 70% ethanol for 1 min, the elytra were removed, and the gut was dissected. Fragments of intestine of about 1 cm were incubated for 48 h (120 rpm, elytra were removed, and the gut was dissected. Fragments were deposited in the Entomological collection Paulo Burnheim (UFAM, Brazil).

To evaluate their ability to ferment D-xylose, a loopful of each isolate was cultured in tubes containing 10 mL of YNBX medium (yeast nitrogen base without amino acids, 6.7 g/L; D-xylose, 40 g/L). After 7 days of incubation at 28°C and 120 rpm, the medium content was centrifuged and the supernatant was analysed by an HPLC system using a Rezex RPM monosaccharide column (300 x 7.8 mm, Ph2+ 8%, Phenomenex). The D-xylose consumption rate (%) was calculated according to the final and initial D-xylose concentrations. Xylitol yield (g 1-1) was calculated by the ratio xylitol produced: D-xylose consumed.

For taxonomic identification, biochemical characterization was performed using kit ID32C (BioMerieux®), according to the manufacturer's instructions. The results were plotted in the online application ApiWeb® (https://apiweb.biomerieux.com) for physiological similarity identification.

Furthermore, the isolates were evaluated by genomic internal transcribed spacer (ITS) and ribosomal gene nucleotide sequences. The DNA was extracted according to Harju et al. [12] and amplified by PCR using primers ITS1 (5’TCC GTA GGT AAT CCT GGC 3’) and ITS4 (5’TCC GGT TAT TGA TAT GC 3’). The PCR products were used to perform a sequencing reaction using a BigDye® kit (Applied Biosystems), and nucleotide sequences were obtained in an Applied Biosystems 3130 Genetic Analyzer® automatic sequencer.

The obtained sequences were compared to the NCBI database (https://www.ncbi.nlm.nih.gov/) using BLAST (Basic Local Alignment Search Tool) and deposited in GenBank. For phylogenetic relationship analysis, nucleotide sequences were aligned using Clustal-W and analysed by neighbour-joining (bootstrap, 2000 replicates), provided by MEGA 6.0 [13]. Nucleotide sequences from the genomic ITS region of *Meyerozyma guilliermondii* (GenBank JN974905), *Trichosporon mycotoxinivorans* (GenBank JX891097), and *Scheffersomyces stipitis* (GenBank GU256745) were included in the phylogenetic tree as reference groups, this last being the external group.

The Neighbour-joining phylogenetic analysis endorsed the conclusion about the three groups that clade *W. saturnus* is close to *Meyerozyma guilliermondii* and *Scheffersomyces stipitis*, whereas *C. tropicalis* and *Geotrichum* sp. are closely related to *Trichosporon mycotoxinivorans*. The phylogenetic tree is presented in Figure 1.

Fermentation tests indicated that none of the isolates produces ethanol using xylene as carbon source. This result was expected because xylose fermentation to ethanol is an uncommon feature, being presented by less than 1% of known yeast species [14]. However, most of them were able to produce xylitol, only isolates 12 and 13 (*Geotrichum* sp.) being unable to do this. The highest yield was observed in isolate 01 (*Geotrichum* sp.), reaching 0.502 g g-1 and consuming 92.6% of the D-xylose. The complete results are presented in Table 3.

4. Discussion

All isolates were able to assimilate D-xylose, a common feature in yeasts able to metabolize SBHH because this is the most abundant monosaccharide in hemicellulose [15]. *Candida* is the most representative, but that occurs because there are a great number of asexual phase (anamorph) species classified in this genus, which is a polyphyletic group [5, 16].

The other genera, *Cryptococcus* and *Debaryomyces*, have remarkable biotechnological potential in incorporation of lipids in their biomass, being reported as oleaginous yeasts [17,18]. Suh and Blackwell [19] describe the genus *Geotrichum* as dimorphic fungi, being anamorphs of the genera *Dipodascus* and *Galactomyces* and growing being yeast-like according to environmental conditions.
| Isolate | GAL | ACT | SAC | NAG | LAT | ARA | CEL | RAF | MAL | TER | 2KG | MDG | SOR | XYL | RIB | GLY | RHA | ERY | MEL | GRT | MLZ | GNT | LVT | MAN | INO | GLU | SBE | GLN | ESC | Species | Similarity (%) |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----------------|
| 01      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. humicola | 98.4%           |
| 02      | +   | −   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. curvatus |                |
| 03      | +   | +   | +   | −   | −   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | D. etchellsii | 79.1%           |
| 04      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. membranifaciens |  |
| 05      | +   | +   | +   | −   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. intermedia |  |
| 06      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. parapsilosis |  |
| 07      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. humicola | 99.2%           |
| 08      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. humicola | 99.7%           |
| 09      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. tropicalis | 53.6%           |
| 10      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. humicola | 99.5%           |
| 11      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | C. famata | 97.7%           |
| 12      | +   | +   | +   | −   | −   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 97.7%           |
| 13      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 97.7%           |
| 14      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 99.5%           |
| 15      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 99.0%           |
| 16      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 83.1%           |
| 17      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 95.4%           |
| 18      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 94.4%           |
| 19      | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | +   | G. capitatum | 95.7%           |

GAL: galactose, ACT: cycloheximide, SAC: sucrose, NAG: N-acetyl glucosamine, LAT: lactic acid, ARA: arabinose, CEL: cellobiose, RAF: raffinose, MAL: maltose, TRE: trehalose, 2KG: 2-keto gluconate, MDG: α-methyl-glucopyranoside, SOR: sorbitol, XYL: xylose, RIB: ribose, GLY: glycerol, RHA: rhamnose, PLE: palatinose, ERY: erythritol, MEL: melibiose, GRT: sodium gluconate, MLZ: melezitose, GNT: potassium gluconate, LVT: levulinic acid, MAN: mannitol, LAC: lactose, INO: inositol, GLU: glucose, SBE: sorbose, GLN: glucosamine, ESC: esculin iron citrate.
Table 2: Identification of isolates according to BLAST result and nucleotide sequence GenBank accession number.

| Isolate | Species                  | Max identity (%) | Query coverage (%) | e-value     | GenBank accession number |
|---------|--------------------------|------------------|--------------------|-------------|--------------------------|
| 01      | Geotrichum sp.           | 93               | 92                 | 1e−119      | KP276644                 |
| 02      | Geotrichum sp.           | 95               | 93                 | 3e−131      | KP276636                 |
| 03      | Candida tropicalis       | 99               | 98                 | 0.0         | KP276645                 |
| 04      | Geotrichum sp.           | 94               | 100                | 1e−121      | KP276637                 |
| 05      | Galactomyces candidum    | 96               | 97                 | 1e−131      | KP276638                 |
| 06      | Geotrichum sp.           | 96               | 99                 | 1e−126      | KP276639                 |
| 07      | Williopsis saturnus      | 99               | 100                | 0.0         | KP257575                 |
| 08      | Candida tropicalis       | 98               | 99                 | 0.0         | KP276646                 |
| 09      | Candida tropicalis       | 99               | 99                 | 0.0         | KP276647                 |
| 10      | Candida tropicalis       | 98               | 99                 | 0.0         | KP276648                 |
| 11      | Candida tropicalis       | 98               | 99                 | 0.0         | KP276649                 |
| 12      | Galactomyces candidum    | 96               | 98                 | 1e−136      | KP276640                 |
| 13      | Galactomyces geotrichum  | 96               | 98                 | 1e−136      | KP276641                 |
| 14      | Geotrichum sp.           | 95               | 100                | 1e−125      | KP276642                 |
| 15      | Geotrichum sp.           | 94               | 100                | 3e−102      | KP288488                 |
| 16      | Williopsis saturnus      | 99               | 100                | 0.0         | KP257574                 |
| 17      | Geotrichum sp.           | 93               | 99                 | 7e−129      | KP288487                 |
| 18      | Williopsis saturnus      | 99               | 100                | 0.0         | KP257573                 |
| 19      | Geotrichum sp.           | 94               | 99                 | 5e−134      | KP276643                 |
| 20      | Candida tropicalis       | 97               | 99                 | 0.0         | KP276650                 |

Table 3: D-xylose consumption rate (%) and xylitol yield of each isolate.

| Isolate | D-xylose consumption rate (%) | Xylitol yield (g g⁻¹) |
|---------|-------------------------------|----------------------|
| 01      | 92.6                          | 0.502                |
| 02      | 29.9                          | 0.210                |
| 03      | 33.4                          | 0.214                |
| 04      | 36.2                          | 0.255                |
| 05      | 30.7                          | 0.186                |
| 06      | 35.6                          | 0.224                |
| 07      | 31.0                          | 0.169                |
| 08      | 33.2                          | 0.180                |
| 09      | 34.0                          | 0.170                |
| 10      | 30.7                          | 0.100                |
| 11      | 100.0                         | 0.339                |
| 12      | 27.5                          | 0.0                  |
| 13      | 18.7                          | 0.0                  |
| 14      | 100.0                         | 0.315                |
| 15      | 100.0                         | 0.326                |
| 16      | 100.0                         | 0.304                |
| 17      | 41.4                          | 0.304                |
| 18      | 100.0                         | 0.341                |
| 19      | 100.0                         | 0.370                |
| 20      | 100.0                         | 0.347                |

All biochemical profile results presented similarity with species able to perform pentose fermentation and/or another process with biotechnological potential. However, according to Barnett [20], biochemical profiles may be used as complementary information but cannot be conclusive for taxonomic identification because they can present high variation, with it being recommended to evaluate genomics data. According to Hou-Rui et al. [21], up to 1% of nucleotide substitution in a ribosomal domain is permitted for strains of a single biological species, rDNA sequence analysis being a simple and reliable tool for taxonomic identification.

The phylogenetic analysis endorses that predicted by Barnett [20], noticeable because clade _W. saturnus_ is composed of isolates with three different biochemical profiles, whereas clade _C. tropicalis_ is composed of five different biochemical profiles (one of those _C. tropicalis_), and clade _Geotrichum_ has eight different biochemical profiles. Furthermore, there were some isolates with the same biochemical profile distributed in all clades, strengthening that hypothesis.

The maximum theoretical yield for xylitol production from D-xylose fermentation is 1.0 g g⁻¹. Despite this, as microbes produce xylitol as a compatible solute, it is excreted in osmotic stress conditions and then consumed as the medium becomes less harsh [22]; common yields range from 40% to 70% [23]. The highest yield value for microbial fermentation is reported by Granström et al. [24] for _Candida_ sp., at 0.85 g g⁻¹.

With _Geotrichum_ sp. (isolate 01) being a wild-type strain with the capability to produce xylitol like some industrial strains, it can be considered a promising xylitol-producing yeast. This is the first work to report xylitol production by wild-type yeast strains associated with beetles from the Central Amazon Rainforest.
5. Conclusion

The yeast community associated with *V. transversus* gut is rich in D-xylose-assimilating and xylitol-producing species, some of which present potential close to industrial strains. *Geotrichum* is a highly representative group in this community.

*Geotrichum* sp. (isolate 01) presents high xylitol yield, reaching about 50% of the maximum theoretical yield, and is a promising xylitol-producing strain.

Subsequent efforts must be concentrated on developing bioprocesses using these isolates.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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