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Xylose-fermenting yeast species found in the wood of southeast Louisiana

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Xylose-fermenting yeast species found in the wood of southeast Louisiana

by

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ABSTRACT

Passalid beetles (*Odontotaenius disjuntus*) have a close relationship with a wide variety of gut microbes, including the yeast *Pichia stipitis* (Saccharomycotina). The beetle excavates galleries within wood, and uses the wood as a habitat and its sole nutritional resource in all stages of the life cycle. *Pichia stipitis* has been found to ferment and assimilate xylose in culture. Xylose fermentation is a rare trait among yeasts, but previous work has shown that most xylose-fermenting yeasts are commonly associated with wood-ingesting beetles. This study was undertaken to determine if yeasts were present in wood of several plant species (*Quercus nigra, Quercus virginiana, and Carya illinoinensis*) in the absence and presence of the beetles in order to determine if beetles acquire the yeast from the wood. Significant findings were 1) xylose-fermenting yeasts were usually absent in wood without galleries; 2) 26 yeast genotypes were present among the 29 strains isolated from wood; 3) of the 3 repeat sequences one genotype was found at different localities in different wood; 4) four strains may represent new species; 5) 7 of 11 genotypes from galleries were *Pichia stipitis*; 3 of 14 genotypes isolated from wood without beetles fermented xylose and are likely undescribed species.

INTRODUCTION

The passalid beetle (*Odontotaenius disjuntus*) excavates galleries in the wood, and it uses the wood both as a sole food source and a habitat. The passalid beetle probably has a symbiotic relationship with a variety of gut microbes (Suh, et al., 2003). The microbes include bacteria, yeast, and at least two different types of protists (Nardi, et al., 2006). This study was performed to determine if wood might be the source of the yeast, *Pichia stipitis* or other xylose-fermenting yeasts, common in the passalid gut.

Xylose is a five carbon sugar contained in the complex polysaccharide, hemicellulose, present in the cell wall of plants. Xylose is the most common sugar found in hardwood and agricultural residues (Jefferies, et al., 2000). Xylose is broken down to xylulose-5-P (Fig. 1). Xylose-5-P is converted by the pentose phosphate pathway to fructose-6-phosphate. Fructose -6-phosphate is the substrate for fermentation or oxidation through respiration. Unlike common sugars such as sucrose and fructose, xylose is not regularly found as a soluble sugar in nature (Jeffries & Jin 2000; Jackson & Nicholson 2002).

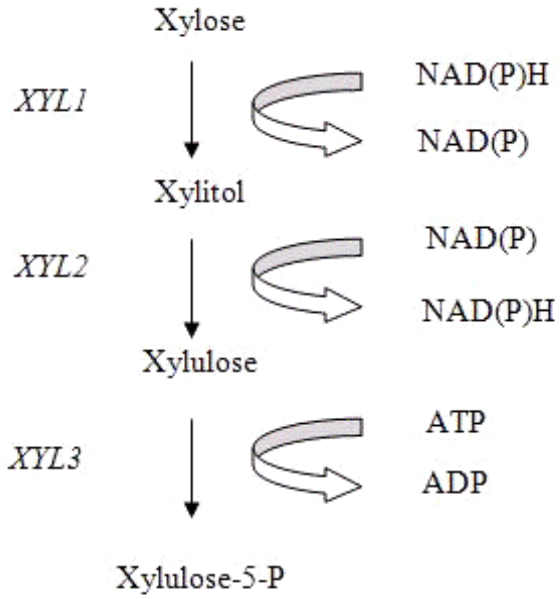


Figure 1. The xylose fermentation pathway begins with the xylose becoming xylitol in yeasts. The enzymes involved in the pathway: Many different enzymes are included in this process: *XYL1* is xylose (Aldose) Reductase; *XYL2* is Xylitol Dehydrogenase; *XYL3* is xylulokinase (Jefferies, et al., 2006).

Pichia stipitis ferments xylose efficiently in vitro, although we know little about its function in vivo. In vitro rates of ethanol production were measured by Jefferies and his colleagues (2000), as a potential source of alcohol as a biofuel. The faster the rate of ethanol production from a substance, such as wood, the more useful the substance will be for biofuel production. The production of ethanol from xylose fermentation is not yet a commercial process (Jefferies, et al., 2000).

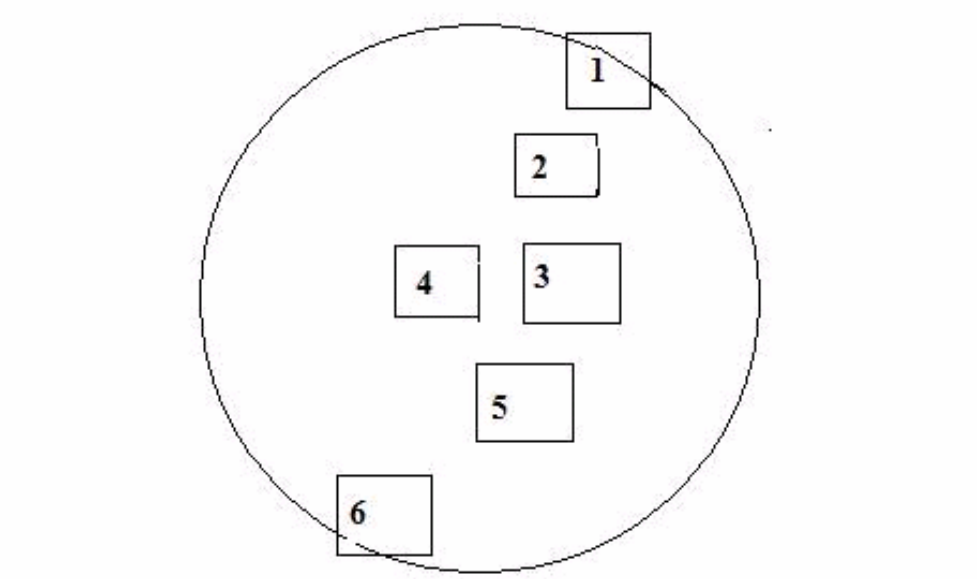
MATERIALS AND METHODS

Site Description

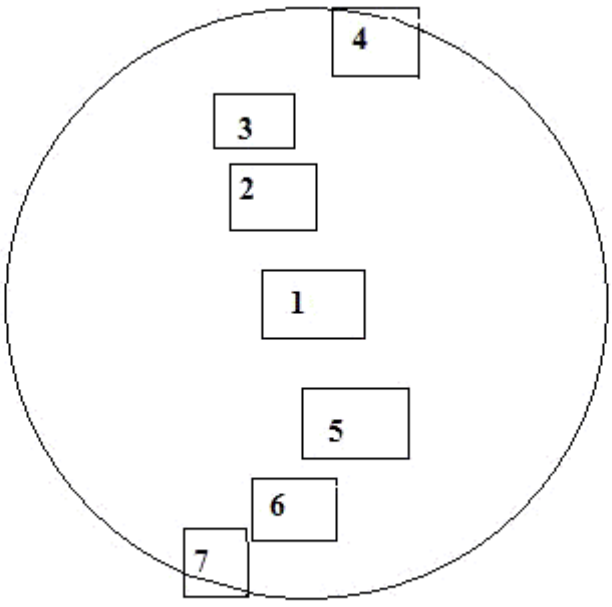
Different types of wood: *Quercus nigra* (water oak), *Quercus virginiana* (live oak), and *Carya illinoinensis* (pecan) were collected, respectively, from LSU Burden Center, on the corner of Highland Road and S. Stadium Drive on the LSU campus, and from Pecan Dr., St. Gabriel, LA (home of Larry Rouse), between September and November, 2007. The dead wood collected was ground and was partially decayed. The live oak and water oak did not contain beetle galleries, and they were collected from fallen branches approximately 10 cm in diameter. However, beetle galleries were present in the badly decomposed pecan wood (Table1).

Specimen Processing

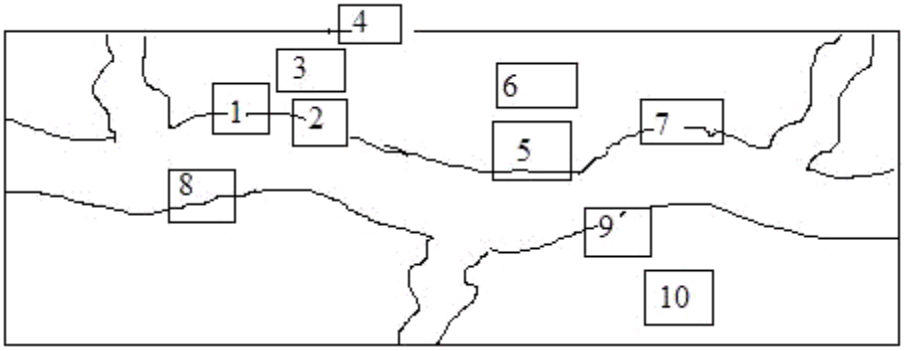
Branches were cut in either a horizontal or vertical direction. Wood chunks that were approximately 1 cm x 1 cm in size were taken from the collected wood. Approximately seven to nine chunks were taken from each branch of wood (Fig. 2). The chunks were placed in a 1.5 mL Eppendorf tube, which contained 1mL of sterile water. The vortex machine was used on the Eppendorf tubes for approximately 30 seconds. The liquid was then spread onto acidified yeast medium agar (AYM), which inhibits bacterial growth. Each isolate was streaked on a separate plate for extraction. The plates were incubated at 25°C. The single colonies were then streaked for purification at least four times on acidified YM agar and incubated at 37°C. The final streak for purification was on YM agar. A total of nine pieces of wood were collected from the various locations. Wood 1 is the water oak, Wood 4 is the live oak, and Wood 9 is the pecan, which was the only wood with beetle galleries. However, the AYM plates from wood samples W 2, 3, 5, 6, 7, 8 became infested with an aggressive fungus and the isolations could not be completed.



Wood 1: *Quercus niger*, LSU Burden Center (horizontal cross section)



Wood 4: *Quercus virginiana*, LSU Campus (horizontal cross-section)



Wood 9: *Carya illinoinensis*, Ascension Parish (vertical cross section)

Figure 2. Wood found in different locations was dissected to isolated yeast.

DNA Analysis

Once the isolates grew on YM agar, DNA extraction was performed using the phenol-choroform method. PCR was performed on the yeast isolates using LS1 (GT ACC CGC TGA ACT TAA G) and LR3 (CCG TGT TTC AAG ACG GG) primers. Each 25.3875 µL PCR reaction contained 2.5 µL 10X reaction buffer, 0.5 µL dNTP (10 mM), .5 µL LR3(10 µM), .5 µl LS1 (10 µM), 1.0 µL diluted (1/100) DNA template, 0.0125 µL Taq DNA polymerase (5U/µl), and 20.375 µL PCR water (Suh, et al., 2007). The PCR program was 95°C X 5 min. (95°C X 1 min. / 55°C X 1 min/ 72 °C X 1 min.) X 35 cycles, 72°C X 10 min. Gel electrophoresis was performed after the PCR reaction to determine if DNA amplification took place. The gel was run using 3 µL of PCR product with 3 µL of 2X loading buffer (dye) and was run at 70-80 V for 30-60 min in 1x TAE buffer. The gel was then stained in EtBr solution (5µL EtBr/100mL water) for 15-20 minutes (Suh, et al., 2007).

The PCR product was purified using the ExoSap PCR Purification Method. Using the remaining PCR product 0.5 µL Exonuclease I as added, 0.5 µL SAP (Shrimp Alkaline Phosphatase), and 1.0 µL water. The program was 37°C X 45 min/ 80°C X 15 min./10 °C hold (Suh, et al., 2007).

PCR purification was followed by the ABI PRISM ^{TM} BigDye terminator cycle

sequencing kit (Table 1). The PCR product (usually 1 µL) was put into 0.2 ml tube. Make total DNA template volume as 2.5 µl by adding PCR water. The reaction mixture included 0.5 µL BigDye termination mix, 1.0 µL LR3 Primer (25ng/µL or 5mM), and 1.0 µL BigDye Buffer. The program was run at (96˚C X 10 sec/ 50˚C X 5 sec/ 60˚C X 4 min) X 25 cycles. Purification of Sequence samples was done using the ethanol precipitation method (Suh, et al., 2007). The samples were sequenced at the LSU Museum of Natural Science. Detailed methods used in this study are available in the laboratory manual on line (Suh, et al., 2008).

Species Identification

BLAST searches ("http://ncbi.nlm.nih.goc/BLAST/") were used to find closest relatives based on rDNA sequences. Gaps and base pairs different from the species in the database and the discovered isolate were recorded.

Assimilation/Fermentation Testing

Xylose assimilation and xylose fermentation tests were run to determine if the yeast isolates were able to utilize and ferment xylose. Xylose assimilation tubes were made with 4.5 mL of deionized H_20 and 0.5 mL of 10X xylose stock solution. The glucose assimilation tubes for positive controls were made with 4.5 mL of deionized H_20 and 0.5 mL of 10X glucose stock solutions and were used as the positive control (Suh, et al., 2007). Durham fermentation tubes were created by measuring 2 g of xylose, 1 g of yeast extract in 100 mL of water. A smaller tube filled with medium was inverted in the test tube and then autoclaved.

Once inoculated, the assimilation tubes were checked at 7 days, 14 days, and 21 days. The criteria used to judge the assimilation tests were (+) if the line drawn on a white card was clearly visible, edges are sharp and not blurry was scored, (++) if the lines are just beginning to become blurry to almost invisible or (+++) if the line was invisible. The fermentation tubes were

checked at 7 days and 21 days. The fermentation tests were rated as (w) for a very small bubble, (+) for a relatively small bubble, (++) indicates a bubble that is less than half of the tube, and (+++) for greater than or equal to half of the tube (Suh, et al., 2007).

RESULTS

Twenty-nine yeast isolations were made from three different types of wood (Table 1). Wood 1, water oak (*Quercus nigra),* and Wood 4, live oak (*Quercus virginiana*), did not have beetle galleries. Each wood sample contained at least two different genotypes. However, not all of them were isolated because some of the cultures were infected with an aggressive filamentous fungus. About 600 bp of LSU rDNA gene was sequenced from PCR products of the wood yeast. Most species found were at least 1 base pair different than the closest taxa found in the BLAST searches, and some may be new species. The yeasts found in Wood 9, pecan wood (*Carya illinoinensis),* had beetle galleries and were the source of the majority of the xylose-fermenting yeast (Table 1).

A total of 9 yeasts fermented xylose by 21 days: W 07-09-15-1-3-2, W 07-10-04-4-2-2, W 07-11-15-9-1-1, W 07-11-15-9-2-1, W 07-11-15-9-2-2, W 07-11-15-9-3-1, W 07-11-15-9-4-1, W 07-11-15-9-5-1, W 07-11-15-9-6-2 (Table 1). One isolate, W 07-10-04-4-6-2, was discovered to be positive for xylose fermentation within 7 days (Table 1).

A total of 26 yeast genotypes were present among the 29 strains isolated from wood (Table 1). Of the 3 repeat genotypes, one genotype was found at different localities in different species of wood. Of the 10 strains of xylose fermenting yeasts found, 3 strains may represent new species that are xylose fermenters. 7 of 11 genotypes from wood with beetle galleries were close to *Pichia stipitis*. Xylose-fermenting yeasts were usually absent in wood without galleries,

but 3 of 14 genotypes isolated from wood without beetle galleries fermented xylose and are likely undescribed species based on base pairs difference between them and known species. Further studies need to be performed with paired samples of wood, which vary in the presence of beetle galleries.

Assimilation and Fermentation

All isolates with sequences near *Pichia stipitis (*W 07-10-04-4-2-2, W 07-11-15-9-1-1, W 07-11-15-9-2-1, W 07-11-15-9-2-2, W 07-11-15-9-3-1, W 07-11-15-9-4-1, W 07-11-15-9-5-1, W 07-11-15-9-6-2) were xylose fermenters by day 21. An isolate (W 07-10-04-4-6-2), 10 base pairs from *Pichia stipitis,* began to ferment xylose by day 7, and a strain near *Candida oleophila* (W 07-09-15-1-3-2) was found to be a xylose fermenter by day 21 (Table 1). All 29 isolates found were able to assimilate xylose.

DISCUSSION

Xylose-fermenting yeasts: Because we were interested in how beetles acquire *Pichia stipitis*, our original goal was to determine if *Pichia stipitis* occurred in wood in the absence of beetles, and within the sampling limitations of not having paired samples mentioned below, we have found *Pichia stipitis* only in association with passalid galleries (Nguyen, et al., 2006). However, the finding of xylose-fermenting yeasts in wood is not unexpected because this trait probably would be useful to free-living yeasts in woody substrates where xylose would be present, especially after the partial decay of wood by wood-decaying fungi. What was surprising, however, was the discovery of three apparently undescribed species of xylose-fermenting yeasts, one of which is an extremely rapid fermenter compared to all other strains studied (Nguyen, et al., 2006; Blackwell, et al., 2007).

Table 1. Database of different yeast isolates found from the LSU Burden Center, the LSU Campus, and Dr. Larry Rouse's home.

Strain	Source	Genotype	Fermentation		Nearest Taxa (no. bp difference/no. gaps difference)
			Xylose		
Isolate			W 1	W 3	
CR 1-1	W 07-09-15-1-1-1 *Quercus niger*, LSU Burden Center	g-1	-	-	*Candida sp.* NRRL YB-1336 (3-0)
CR 1-2	W 07-09-15-1-1-2 *Quercus niger*, LSU Burden Center	g-2	-	-	*Candida sp.* NRRL YB-1336 (0-0)
CR 1-3	W 07-09-15-1-2-1 *Quercus niger*, LSU Burden Center	g-3	NA	NA	*Candida sp.* BG-02-7-13-013A-1-1-1(3-2)
CR 1-4	W 07-09-15-1-3-1 *Quercus niger*, LSU Burden Center	g-4	-	-	*Candida oleophila* strain CBS 4704 (3-1)
CR 1-5	W 07-09-15-1-3-2 *Quercus niger*, LSU Burden Center	g-5	-	+++	*Candida oleophila* strain CBS 4704 (6-2)
CR 1-6	W 07-09-15-1-4-1 *Quercus niger*, LSU Burden Center	g-6	NA	NA	*Candida sp.* NRRL YB-1835 (6-2)
CR 1-7	W 07-09-15-1-5-1 *Quercus niger*, LSU Burden Center	g-7	NA	NA	*Candida sp.* NRRL YB-1336 (3-2)
CR 1-8	W 07-09-15-1-5-3 *Quercus niger*, LSU Burden Center	g-8	-	-	*Candida sp.* NRRL YB-1835 (1-1)
CR 4-1	W 07-10-04-4-1-3 *Quercus virginiana*, LSU Campus	g-8	-	-	*Candida sp.* NRRL YB-1835 (1-1)
CR 4-2	W 07-10-04-4-2-2 *Quercus virginiana*, LSU Campus	g-9	-	+++	*Pichia stipitis* CBS 6054 (14-2)
CR 4-3	W 07-10-04-4-2-3 *Quercus virginiana*, LSU Campus	g-10	-	-	*Candida sp.* BG-02-17-9-009B-1-1 (1-1)
CR 4-4	W 07-10-04-4-3-1 *Quercus virginiana*, LSU Campus	g-10	-	-	*Candida sp.* BG-02-7-17-009B-1-1 (1-1)
CR 4-5	W 07-10-04-4-4-1 *Quercus virginiana*, LSU Campus	g-11	-	-	*Candida sp.* BG-02-7-20-020A-1 (2-2)
CR 4-6	W 07-10-04-4-5-2 *Quercus virginiana*, LSU Campus	g-12	-	-	*Candida sp.* BG-02-7-13-013A-1-1-1(2-1)
CR 4-7	W 07-10-04-4-6-1 *Quercus virginiana*, LSU Campus	g-13	-	-	*Candida sp.* BG-02-7-13-013A-1-1-1(3-2)
CR 4-8	W 07-10-04-4-6-2 *Quercus virginiana*, LSU Campus	g-14	+	+++	*Pichia stipitis* CBS 6054 (10-1)
CR 4-9	W 07-10-04-4-7-2 *Quercus virginiana*, LSU Campus	g-15	-	-	*Candida anneliseae* strain BG-99-8-11-1-2-2 (1-1)
CR 9-1	W 07-11-15-9-1-1 *Carya illinoinensis* from Ascension	g-16	-	+++	*Pichia stipitis* CBS 6054 (2-0)
CR 9-2	W 07-11-15-9-2-1 *Carya illinoinensis* from Ascension	g-17	-	+++	*Pichia stipitis* CBS 6054 (1-1)
CR 9-3	W 07-11-15-9-2-2 *Carya illinoinensis* from Ascension	g-18	-	+++	*Pichia stipitis* CBS 6054 (3-2)
CR 9-4	W 07-11-15-9-3-1 *Carya illinoinensis* from Ascension	g-19	-	+++	*Pichia stipitis* CBS 6054 (2-1)
CR 9-5	W 07-11-15-9-4-1 *Carya illinoinensis* from Ascension	g-20	-	+++	*Pichia stipitis* CBS 6054 (3-2)
CR 9-6	W 07-11-15-9-5-1 *Carya illinoinensis* from Ascension	g-21	-	+++	*Pichia stipitis* CBS 6054 (4-2)
CR 9-7	W 07-11-15-9-6-2 *Carya illinoinensis* from Ascension	g-22	-	+++	*Pichia stipitis* CBS 6054 (2-1)
CR 9-8	W 07-11-15-9-7-2 *Carya illinoinensis* from Ascension	g-23	-	-	*Candida sp.* BG 02-7-18-002A-4-2 (0-0)
CR 9-9	W 07-11-15-9-8-1 *Carya illinoinensis* from Ascension	g-24	-	-	*Candida athensensis* strain BG02-7-13-014-3-1 (1-0)
CR 9-10	W 07-11-15-9-8-2 *Carya illinoinensis* from Ascension	g-25	-	-	*Candida athensensis* strain BG02-7-13-014-3-1 (1-0)
CR 9-11	W 07-11-15-9-9-2 *Carya illinoinensis* from Ascension	g-26	-	-	*Candida sp* BG 01-7-21-010A-4-4 (1-1)
CR 9-12	W 07-11-15-9-9-3 *Carya illinoinensis* from Ascension	g-23	-	-	*Candida sp.* BG 02-7-18-002A-4-2 (0-0)

Biodiversity: The finding that so many different yeast genotypes were present among the 29 strains isolated from wood is of interest as an indication of the great diversity of yeasts and the lack of diversity among GenBank database sequences. Although D1/D2 sequence of the type of all described species of yeasts have been submitted to GenBank, this study indicates that the database is a long way from saturation, especially when sequences vary even slightly from the type (Zhang, et al., 2003). Of the three pairs of repeat sequences discovered two were isolated nearby the strain with the identical sequence and were widespread in the wood sample. The third pair was distinctive because it was collected from two species of oak in localities about 5 miles apart.

The study has provided some significant findings, but one flaw in its execution was the failure to sample beetle-infested and beetle-free wood from the same plant species. Subsequent studies should investigate the use of paired samples, preferably of the same size and stage of decomposition. It should be noted, however, that it is often difficult to find adequate amounts of white-rotted wood that has not been invaded by passalid beetles.

Additionally, because xylose fermentation has attracted the interest of those who have attempted to use microbes for biofuel production (Jefferies, et al., 2006), experiments could be done to look at the rate of xylose fermentation of the different strains, because the greater the rate of fermentation, the more efficiently ethanol could be produce.

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Appendix A. Complete database of different yeast isolates found from the LSU Burden Center, the LSU Campus, and Dr. Larry Rouse's home.

Strain Designation		Source	Galleries	Nearest Taxa (no. bp diffference/no. gaps difference)
Isolate	LSU			
CR 1-1	**W 07-09-15-1-1-1**	***Quercus niger*, LSU Burden Center**	**No**	**Candida sp. NRRL YB-1336 (3-0)**
				Candida sp. NRRL YB-1473 (6-1)
				Candida sp. NRRL Y-27117 (9-4)
				Candida sp. NRRL YB-2263 (14-5)
				Candida novakii DQ 438196 (15-4)
CR 1-2	**W 07-09-15-1-1-2**	***Quercus niger*, LSU Burden Center**	**No**	**Candida sp. NRRL YB-1336 (0-0)**
				Candida sp. NRRL YB-1473 (3-0)
				Candida sp. NRRL Y-27117 (7-3)
				Candida sp. NRRL YB-2263 (12-4)
				Candida novakii DQ 438196 (12-3)
CR 1-3	**W 07-09-15-1-2-1**	***Quercus niger*, LSU Burden Center**	**No**	**Candida sp. BG-02-7-13-013A-1-1-1(3-2)**
				Stephanoascus smithiae DQ 438218 (2-2)
CR 1-4	**W 07-09-15-1-3-1**	***Quercus niger*, LSU Burden Center**	**No**	**Candida oleophila strain CBS 4704 (3-1)**
				Candida oleophila strain CBS 4410 (3-1)
CR 1-5	**W 07-09-15-1-3-2**	***Quercus niger*, LSU Burden Center**	**No**	**Candida oleophila strain CBS 4704 (6-2)**
				Pichia stipitis CBS 6054 (17-3)
CR 1-6	**W 07-09-15-1-4-1**	***Quercus niger*, LSU Burden Center**	**No**	**Candida sp. NRRL YB-1835 (6-2)**
				Candida sp. NRRL YB-3827 (9-2)
				Candida valdiviana U45835 (22-10)
CR 1-7	**W 07-09-15-1-5-1**	***Quercus niger*, LSU Burden Center**	**No**	**Candida sp. NRRL YB-1336 (3-2)**
				Candida sp. NRRL YB-1473 (6-2)
				Candida sp. NRRL Y-2717 (10-5)
				Candida sp. NRRL YB-2263 (15-6)
				Candida novakii DQ 438196 (15-5)
CR 1-8	**W 07-09-15-1-5-3**	***Quercus niger*, LSU Burden Center**	**No**	**Candida sp. NRRL YB-1835 (1-1)**
				Candida sp. NRRL YB-3827 (4-1)
				Candida valdiviana U45835 (18-10)

Strain Designation		Nearest Taxa (no. bp diffference/no. gaps difference)	Fermentation		Assimilation						Description
			Xylose		Xylose			Glucose			
Isolate	LSU		W 1	W 3	W 1	W 2	W 3	W 1	W 2	W 3	
CR 1-1	**W 07-09-15-1-1-1**	**Candida sp. NRRL YB-1336 (3-0)**	-	-	++	++	++	++	++	++	g-1
		Candida sp. NRRL YB-1473 (6-1)									
		Candida sp. NRRL Y-27117 (9-4)									
		Candida sp. NRRL YB-2263 (14-5)									
		Candida novakii DQ 438196 (15-4)									
CR 1-2	**W 07-09-15-1-1-2**	**Candida sp. NRRL YB-1336 (0-0)**	-	-	++	++	++	++	++	++	g-2
		Candida sp. NRRL YB-1473 (3-0)									
		Candida sp. NRRL Y-27117 (7-3)									
		Candida sp. NRRL YB-2263 (12-4)									
		Candida novakii DQ 438196 (12-3)									
CR 1-3	**W 07-09-15-1-2-1**	**Candida sp. BG-02-7-13-013A-1-1-1(3-2)**	NA	NA	NA	NA	NA	NA	NA	NA	g-3
		Stephanoascus smithiae DQ 438218 (2-2)									
CR 1-4	**W 07-09-15-1-3-1**	**Candida oleophila strain CBS 4704 (3-1)**	-	-	+	+	+	+	+	+	g-4
		Candida oleophila strain CBS 4410 (3-1)									
CR 1-5	**W 07-09-15-1-3-2**	**Candida oleophila strain CBS 4704 (6-2)**	-	+++	++	++	++	++	++	++	g-5
		Pichia stipitis CBS 6054 (17-3)									
CR 1-6	**W 07-09-15-1-4-1**	**Candida sp. NRRL YB-1835 (6-2)**	NA	NA	NA	NA	NA	NA	NA	NA	g-6
		Candida sp. NRRL YB-3827 (9-2)									
		Candida valdiviana U45835 (22-10)									
CR 1-7	**W 07-09-15-1-5-1**	**Candida sp. NRRL YB-1336 (3-2)**	NA	NA	NA	NA	NA	NA	NA	NA	g-7
		Candida sp. NRRL YB-1473 (6-2)									
		Candida sp. NRRL Y-2717 (10-5)									
		Candida sp. NRRL YB-2263 (15-6)									
		Candida novakii DQ 438196 (15-5)									
CR 1-8	**W 07-09-15-1-5-3**	**Candida sp. NRRL YB-1835 (1-1)**	-	-	++	++	++	++	++	++	g-8
		Candida sp. NRRL YB-3827 (4-1)									
		Candida valdiviana U45835 (18-10)									

Strain Designation		Source	Galleries	Nearest Taxa (no. bp diffference/no. gaps difference)
Isolate	LSU			
CR 4-1	**W 07-10-04-4-1-3**	***Quercus virginiana*, LSU Campus**	**No**	**Candida sp. NRRL YB-1835 (1-1)**
				Candida sp. NRRL YB-3827 (4-1)
				Candida valdiviana U45835 (18-10)
CR 4-2	**W 07-10-04-4-2-2**	***Quercus virginiana*, LSU Campus**	**No**	**Pichia stipitis CBS 6054 (14-2)**
				Candida oleophila strain CBS 4704 (3-1)
CR 4-3	**W 07-10-04-4-2-3**	***Quercus virginiana*, LSU Campus**	**No**	**Candida sp. BG-02-17-9-009B-1-1 (1-1)**
				Candida sp. BG-02-7-20-020A-1 (1-1)
				Trichomonascus petasosporus DQ 442691 (1-1)
CR 4-4	**W 07-10-04-4-3-1**	***Quercus virginiana*, LSU Campus**	**No**	**Candida sp. BG-02-7-17-009B-1-1 (1-1)**
				Candida sp. BG-02-7-20-020A-2-1 (1-1)
				Trichomonascus petasosporus DQ 442691 (1-1)
CR 4-5	**W 07-10-04-4-4-1**	***Quercus virginiana*, LSU Campus**	**No**	**Candida sp. BG-02-7-20-020A-1 (2-2)**
				Candida sp. BG-02-7-17-009B-1-1 (2-2)
				Trichomonascus petasosporus DQ442691 (2-2)
CR 4-6	**W 07-10-04-4-5-2**	***Quercus virginiana*, LSU Campus**	**No**	**Candida sp. BG-02-7-13-013A-1-1-1(2-1)**
				Stephanoascus Smithiae DQ438218 (2-2)
CR 4-7	**W 07-10-04-4-6-1**	***Quercus virginiana*, LSU Campus**	**No**	**Candida sp. BG-02-7-13-013A-1-1-1(3-2)**
				Stephanoascus Smithiae DQ438218 (4-3)
CR 4-8	**W 07-10-04-4-6-2**	***Quercus virginiana*, LSU Campus**	**No**	**Pichia stipitis CBS 6054 (10-1)**
				Candida oleophila strain CBS 4704 (2-1)
CR 4-9	W 07-10-04-4-7-2	***Quercus virginiana*, LSU Campus**	No	*Candida anneliseae* strain BG-99-8-11-1-2-2 (1-1)
				Candida anneliseae strain BG-02-7-17-011F-2-2 (1-1)

Strain Designation		Nearest Taxa (no. bp diffference/no. gaps difference)	Fermentation		Assimilation						Description
			Xylose		Xylose			Glucose			
Isolate	LSU		W 1	W 3	W 1	W 2	W 3	W 1	W 2	W 3	
CR 4-1	W 07-10-04-4-1-3	**Candida sp. NRRL YB-1835 (1-1)**	-	-	++	++	++	++	++	++	g-8
		Candida sp. NRRL YB-3827 (4-1)									
		Candida valdiviana U45835 (18-10)									
CR 4-2	W 07-10-04-4-2-2	**Pichia stipitis CBS 6054 (14-2)**	-	+++	++	++	++	++	++	++	g-9
		Candida oleophila strain CBS 4704 (3-1)									
CR 4-3	W 07-10-04-4-2-3	**Candida sp. BG-02-17-9-009B-1-1 (1-1)**	-	-	++	++	+++	++	++	++	g-10
		Candida sp. BG-02-7-20-020A-1 (1-1)									
		Trichomonascus petasosporus DQ 442691 (1-1)									
CR 4-4	W 07-10-04-4-3-1	**Candida sp. BG-02-7-17-009B-1-1 (1-1)**	-	-	++	++	+++	++	++	++	g-10
		Candida sp. BG-02-7-20-020A-2-1 (1-1)									
		Trichomonascus petasosporus DQ 442691 (1-1)									
CR 4-5	W 07-10-04-4-4-1	**Candida sp. BG-02-7-20-020A-1 (2-2)**	-	-	++	++	+++	++	++	++	g-11
		Candida sp. BG-02-7-17-009B-1-1 (2-2)									
		Trichomonascus petasosporus DQ442691 (2-2)									
CR 4-6	W 07-10-04-4-5-2	**Candida sp. BG-02-7-13-013A-1-1-1(2-1)**	-	-	++	++	+++	++	++	++	g-12
		Stephanoascus Smithiae DQ438218 (2-2)									
CR 4-7	W 07-10-04-4-6-1	**Candida sp. BG-02-7-13-013A-1-1-1(3-2)**	-	-	++	++	+++	++	++	+++	g-13
		Stephanoascus Smithiae DQ438218 (4-3)									
CR 4-8	W 07-10-04-4-6-2	**Pichia stipitis CBS 6054 (10-1)**	+	+++	++	++	+++	++	+++	+++	g-14
		Candida oleophila strain CBS 4704 (2-1)									
CR 4-9	W 07-10-04-4-7-2	***Candida anneliseae* strain BG-99-8-11-1-2-2 (1-1)**	-	-	+	++	+++	++	+++	+++	g-15
		Candida anneliseae strain BG-02-7-17-011F-2-2 (1-1)									

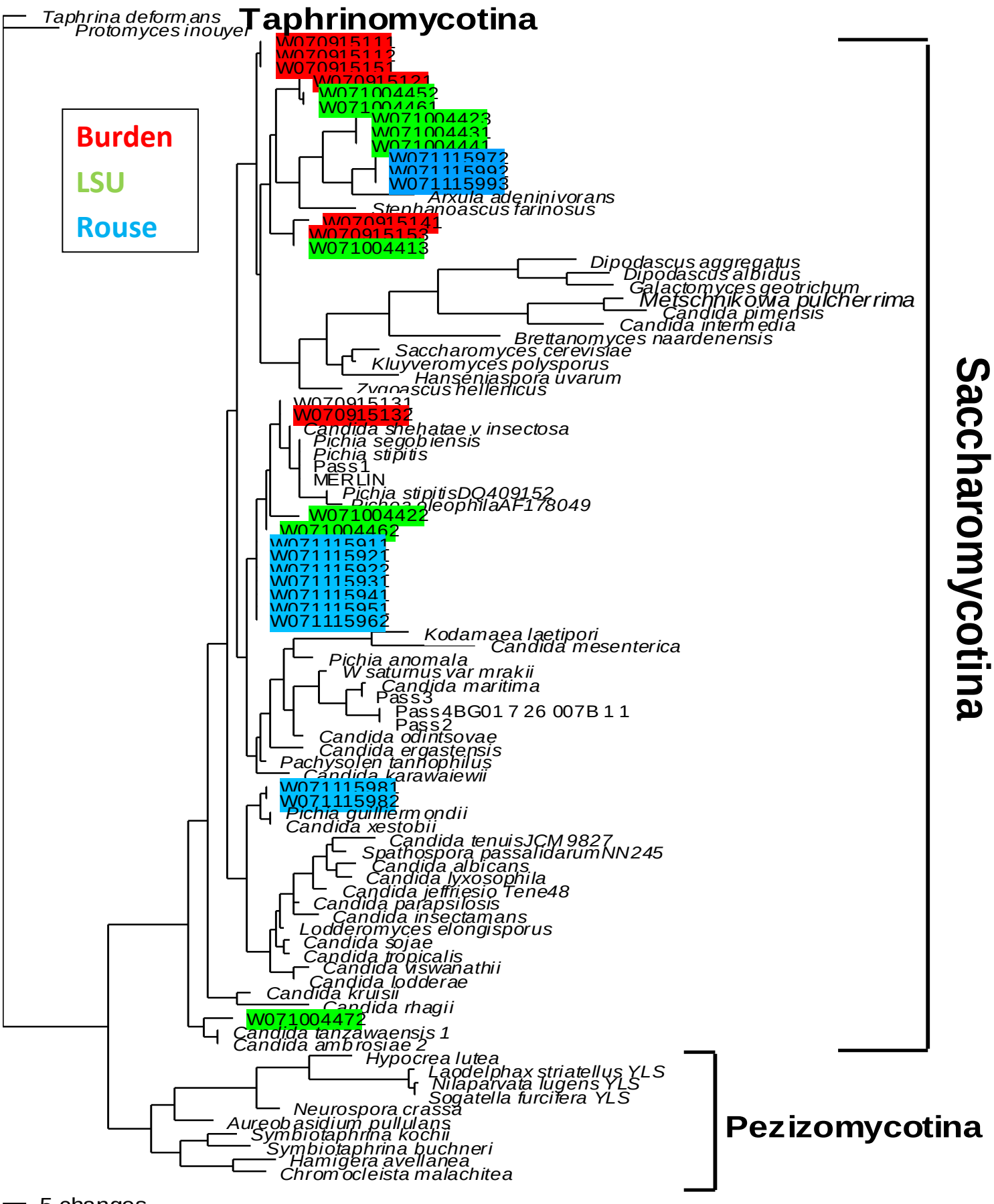
Strain Designation		Source	Galleries	Nearest Taxa (no. bp diffference/no. gaps difference)
Isolate	LSU			
CR 9-1	**W 07-11-15-9-1-1**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (2-0))**
				Pichia stipitis strain CECT1922 (1-0)
				Pichia stipitis KS-42-W2 (2-0)
				Candida sp. BG 02-2-11-6-5 (1-0)
CR 9-2	**W 07-11-15-9-2-1**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (1-1)**
				Pichia stipitis CECT 1922 (1-1)
				Candida sp. BG02-7-14-003-2-1
CR 9-3	**W 07-11-15-9-2-2**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (3-2)**
				Pichia stipitis CECT 1922 (1-1)
				Candida sp. BG 02-7-14-003-2-1 (3-2)
CR 9-4	**W 07-11-15-9-3-1**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (2-1)**
				Pichia stipitis strain CECT 1922 (1-1)
				Candida sp. BG 02-7-14-003-2-1 (1-1)
CR 9-5	**W 07-11-15-9-4-1**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (3-2)**
				Pichia stipitis strain CECT1922 (1-1)
				Pichia stipitis KS-42-W2 (2-1)
				Pichia segobiensis strain CECT 10210 (3-3)
				Candida sp. BG 02-2-11-6-5 (1-1)
CR 9-6	**W 07-11-15-9-5-1**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (4-2)**
				Pichia stipitis strain CECT1922 (2-1)
				Pichia stipitis KS-42-W2 (3-1)
				Pichia segobiensis strain CECT 10210 (4-3)
				Candida sp. BG 02-2-11-6-5 (2-1)

Strain Designation		Nearest Taxa (no. bp diffference/no. gaps difference)	Fermentation		Assimilation						Description
			Xylose		Xylose			Glucose			
Isolate	LSU		W 1	W 3	W 1	W 2	W 3	W 1	W 2	W 3	
CR 9-1	W 07-11-15-9-1-1	**Pichia stipitis CBS 6054 (2-0)**	-	+++	++	+++	+++	++	+++	+++	g-16
		Pichia stipitis strain CECT1922 (1-0)									
		Pichia stipitis KS-42-W2 (2-0)									
		Candida sp. BG 02-2-11-6-5 (1-0)									
CR 9-2	W 07-11-15-9-2-1	**Pichia stipitis CBS 6054 (1-1)**	-	+++	++	+++	+++	++	++	+++	g-17
		Pichia stipitis CECT 1922 (1-1)									
		Candida sp. BG02-7-14-003-2-1									
CR 9-3	W 07-11-15-9-2-2	**Pichia stipitis CBS 6054 (3-2)**	-	+++	++	+++	+++	++	+++	+++	g-18
		Pichia stipitis CECT 1922 (1-1)									
		Candida sp. BG 02-7-14-003-2-1 (3-2)									
CR 9-4	W 07-11-15-9-3-1	**Pichia stipitis CBS 6054 (2-1)**	-	+++	++	++	+++	++	+++	+++	g-19
		Pichia stipitis strain CECT 1922 (1-1)									
		Candida sp. BG 02-7-14-003-2-1 (1-1)									
CR 9-5	W 07-11-15-9-4-1	**Pichia stipitis CBS 6054 (3-2)**	-	+++	++	+++	+++	++	++	+++	g-20
		Pichia stipitis strain CECT1922 (1-1)									
		Pichia stipitis KS-42-W2 (2-1)									
		Pichia segobiensis strain CECT 10210 (3-3)									
		Candida sp. BG 02-2-11-6-5 (1-1)									
CR 9-6	W 07-11-15-9-5-1	**Pichia stipitis CBS 6054 (4-2)**	-	+++	++	++	+++	++	++	+++	g-21
		Pichia stipitis strain CECT1922 (2-1)									
		Pichia stipitis KS-42-W2 (3-1)									
		Pichia segobiensis strain CECT 10210 (4-3)									
		Candida sp. BG 02-2-11-6-5 (2-1)									

Strain Designation		Source	Galleries	Nearest Taxa (no. bp diffference/no. gaps difference)
Isolate	LSU			
CR 9-7	**W 07-11-15-9-6-2**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Pichia stipitis CBS 6054 (2-1)**
				Pichia stipitis strain CECT1922 (2-1)
				Pichia stipitis KS-42-W2 (3-1)
				Pichia segobiensis strain CECT 10210 (4-3)
				Candida sp. BG 02-2-11-6-5 (2-1)
CR 9-8	**W 07-11-15-9-7-2**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Candida sp. BG 02-7-18-002A-4-2 (0-0)**
				Candida sp. BG 02-7-15-009-2-1 (0-0)
				Candida sp. BG 01-7-21-010A-4-1 (0-0)
				Candida sp. BG 01-7-26-005-A-2- (4-0)
CR 9-9	**W 07-11-15-9-8-1**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Candida athensensis strain BG02-7-13-014-3-1 (1-0)**
				Candida athensensis strain BG02-5-23-003I-4 (1-0)
				Candida athensensis strain BG99-8-11-1-C1(4-0)
				Candida xesobii AM160626 (12-2)
CR 9-10	**W 07-11-15-9-8-2**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Candida athensensis strain BG02-7-13-014-3-1 (1-0)**
				Candida athensensis strain BG02-5-23-003I-4 (1-0)
				Candida xesobii AM160626 (13-2)
CR 9-11	**W 07-11-15-9-9-2**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Candida sp BG 01-7-21-010A-4-4 (1-1)**
				Candida sp. BG 02-7-18-002A-4-2 (1-1)
				Candida sp. BG 02-7-15-009-2-2 (1-1)
				Candida sp. BG 01-7-26-005A-2-1 (5-1)
				Candida sp. BG02-7-15-013-2-1 (16-4)
				Arxula adeninivorans IGS Z50840 (44-19)
CR 9-12	**W 07-11-15-9-9-3**	***Carya illinoinensis* from Ascension Parish**	**Yes**	**Candida sp. BG 02-7-18-002A-4-2 (0-0)**
				Candida sp. BG 02-7-15-009-2-2 (0-0)
				Candida sp BG 01-7-21-010A-4-4 (0-0)
				Candida sp. BG 01-7-26-005A-2-1 (4-0)
				Candida sp. BG02-7-15-013-2-1 (15-3)

Strain Designation		Nearest Taxa (no. bp diffference/no. gaps difference)	Fermentation		Assimilation						Description
			Xylose		Xylose			Glucose			
Isolate	LSU		W 1	W 3	W 1	W 2	W 3	W 1	W 2	W 3	
CR 9-7	**W 07-11-15-9-6-2**	**Pichia stipitis CBS 6054 (2-1)**	-	+++	++	+++	+++	++	++	+++	g-22
		Pichia stipitis strain CECT1922 (2-1)									
		Pichia stipitis KS-42-W2 (3-1)									
		Pichia segobiensis strain CECT 10210 (4-3)									
		Candida sp. BG 02-2-11-6-5 (2-1)									
CR 9-8	**W 07-11-15-9-7-2**	**Candida sp. BG 02-7-18-002A-4-2 (0-0)**	-	-	++	++	+++	++	+++	+++	g-23
		Candida sp. BG 02-7-15-009-2-1 (0-0)									
		Candida sp. BG 01-7-21-010A-4-1 (0-0)									
		Candida sp. BG 01-7-26-005-A-2- (4-0)									
CR 9-9	**W 07-11-15-9-8-1**	**Candida athensensis strain BG02-7-13-014-3-1 (1-0)**	-	-	++	+++	+++	++	+++	+++	g-24
		Candida athensensis strain BG02-5-23-003I-4 (1-0)									
		Candida athensensis strain BG99-8-11-1-C1(4-0)									
		Candida xesobii AM160626 (12-2)									
CR 9-10	**W 07-11-15-9-8-2**	**Candida athensensis strain BG02-7-13-014-3-1 (1-0)**	-	-	++	++	++	++	++	+++	g-25
		Candida athensensis strain BG02-5-23-003I-4 (1-0)									
		Candida xesobii AM160626 (13-2)									
CR 9-11	**W 07-11-15-9-9-2**	**Candida sp BG 01-7-21-010A-4-4 (1-1)**	-	-	++	++	++	++	++	+++	g-26
		Candida sp. BG 02-7-18-002A-4-2 (1-1)									
		Candida sp. BG 02-7-15-009-2-2 (1-1)									
		Candida sp. BG 01-7-26-005A-2-1 (5-1)									
		Candida sp. BG02-7-15-013-2-1 (16-4)									
		Arxula adeninivorans IGS Z50840 (44-19)									
CR 9-12	**W 07-11-15-9-9-3**	**Candida sp. BG 02-7-18-002A-4-2 (0-0)**	-	-	++	++	+++	++	++	+++	g-23
		Candida sp. BG 02-7-15-009-2-2 (0-0)									
		Candida sp BG 01-7-21-010A-4-4 (0-0)									
		Candida sp. BG 01-7-26-005A-2-1 (4-0)									
		Candida sp. BG02-7-15-013-2-1 (15-3)									

Appendix B: Phylogenetic Tree Results



Appendix C. Lab: Endosymbiosis and Xylose Fermentation in Yeast

Background:

The passalid beetle (*Odontotaenius disjuntus*) creates galleries in dead, partially rotted hardwood trees, and the beetles use the wood as both a food source and a habitat. Similar symbiotic systems have been identified in the gut of termites and wood roaches. Passalid beetles may have an endosymbiotic relationship with different microbes that are consistently found within their gut (Suh, et al., 2003). Biologists believe that microbes help the beetles with digestion and provide different nutrients and energy. The microbes found include bacteria, yeast, and at least two different types of protists (Nardi, et al., 2006). Also, microbes found in the gut of some organisms, including beetles, are the first "line of defense" because they detoxify plant toxins (Berkov, et al., 2007).

Fermentation is the process that many yeasts use to obtain energy and improve the quality of their food. Yeasts and other organisms can ferment different types of sugars: glucose, fructose, and xylose to generate ATP, an energy compound. Anaerobic bacteria and other anaerobic organisms ferment sugars without the presence of O_2. Fermentation can be detected by the formation of $C0_2$ using Durham fermentation tubes or carbon dioxide sensors. Fermentation is a common process and is involved in the processing of many common food items including the rising of bread and producing alcoholic beverages including beer and wine.

Aerobic Fermentation:
Sugar (glucose, xylose, etc.) + Oxygen→ Alcohol (ethanol) + Carbon Dioxide + Energy (ATP)

The ability to ferment xylose is rare among yeasts. Xylose is a five carbon sugar contained in one of the complex polysaccharide, hemicellulose molecules that make up the plant cell wall, and it is the most common sugar available in hardwood and agricultural residues (Jefferies, et al., 2000). Unlike common sugars such as sucrose and fructose, xylose is not regularly found as a soluble sugar in nature (Jeffries & Jin 2000; Jackson & Nicholson 2002).

The yeast, *Pichia stipitis,* ferments and assimilates xylose in the gut of the passalid beetle. It has a high rate of ethanol production and has been found to be the best species to ferment xylose (Jefferies, et al, 2000). Ethanol is an important product of fermentation because

it could be used as a source of commercial energy to replace petroleum products as biofuels. The faster the rate of ethanol production from a substance, such as wood, the more useful the substance will be for energy. The production of ethanol by xylose fermentation, however, is not yet a commercial process (Jefferies, et al., 2006).

The two types of yeast that will be characterized for xylose-fermentation are *Candida sp.* and *Pichia stipitis*. *Candida sp.* does not ferment xylose. However, *Pichia stipitis* has the rare ability to ferment xylose within at least 21 days. A Durham fermentation tube is used to test for fermentation of the yeasts. A Durham fermentation tube consists of a small tube inverted within a larger test tube; both tubes are filled with media. If fermentation occurs, bubbles of CO_2 rise to displace the liquid within the smaller tube.

Pre Questions:

1) Explain the endosymbiotic relationship of the yeasts and beetles.

_____

_____

2) List two other examples endosymbiotic relationships in nature.

_____

_____

3) What is xylose? Why is it important to plants?

_____

_____

4) List at least two examples of fermentation that are important to the daily lives of many people.

_____

_____

5) What gas is released during the fermentation process?

_____

Name_____ **Date_____**

Lab: Endosymbiosis and Xylose Fermentation in Yeast

Supplies:
Cultures of *Candida sp*. and *Pichia stipitis* (in Petri Dish grown on Yeast Media Agar)
Sterile Durham Fermentation Tubes
Small Sterile Loops

Through this lab, you will determine what culture is *Candida sp*. and *Pichia stipitis.*

MYSTERY: Is *Pichia stipitis*, the xylose fermenting yeast, Yeast A or Yeast B?

Procedure:

1. Take **Yeast A** and remove the Parafilm.
2. Use the small sterile loop to take a colony of Yeast A from the culture.
4. Open the Sterile Durham fermentation tube
3. Take the sterile loop containing the yeast and gently spread the yeast on the liquid line inside the Durham Fermentation tube.
4. Close the Durham fermentation tube and label the tube Yeast A.

**Be careful not to disturb the small inverted tube inside the Durham fermentation tube.

5. Repeat Steps 1-4 for **Yeast B.**
6. Incubate at 25 ºC (room temperature).
7. Check the tubes on Day 7, Day 14, and Day 21 after inoculation.
8. Record the findings in the Data Table.

Use the following criteria to evaluate the bubble inside the small inverted tube:

w	very small bubble
+	relatively small bubble
++	< 1/2 of tube
+++	> 1/2 of tube

9. After Day 21, determine what yeast is *Candida sp.* and *Pichia stipitis.*

Data Table

	Determined Yeast	Day 0	Day 7	Day 14	Day 21
Yeast A	_____				
Yeast B	_____				

Post-Questions:

1) What yeast fermented xylose (Yeast A or B)? _____

2) What is the evidence for your answer?

_____
_____

3) What would happen if you inoculated a fermentation tube containing only water? Explain.

_____
_____
_____

4) What possible benefits do you see through xylose fermentation?

_____
_____

Extended Projects:

-Using baked (sterile) wood and expose it to beetles
-Placing baked (sterile) and unbaked wood in fermentation tubes and making observations

Changing Conditions of Fermentation:
-Light and dark conditions
-Different temperatures
-Different pH

References

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Suh, S., Nguyen, N.H., Blackwell, M. (2005) Nine new *Candida* species near *C. membranifaciens* isolated from insects. *Mycological Research*, 109, 1045-1056.

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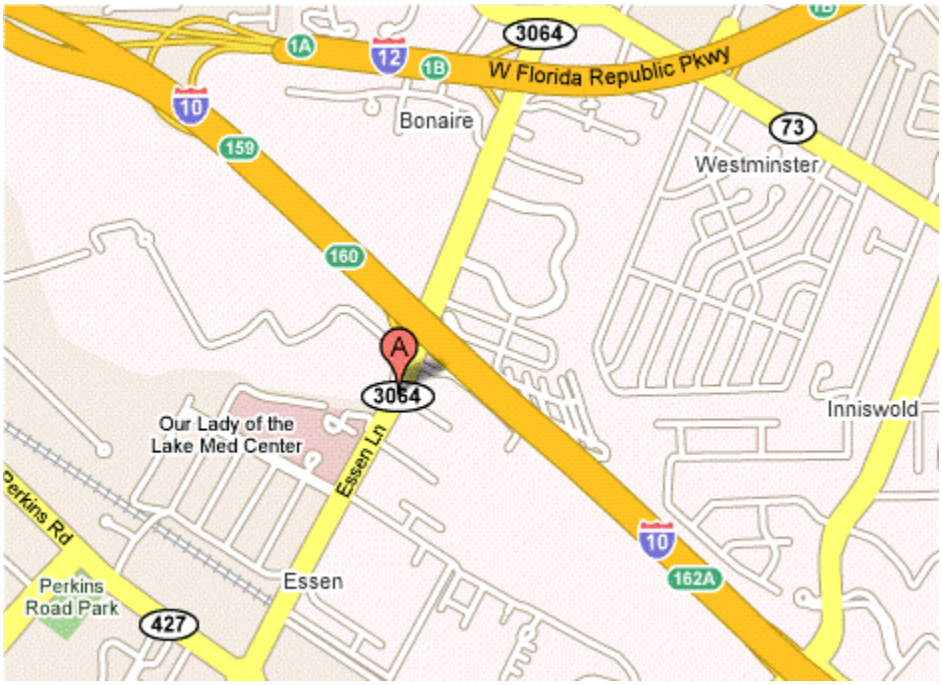
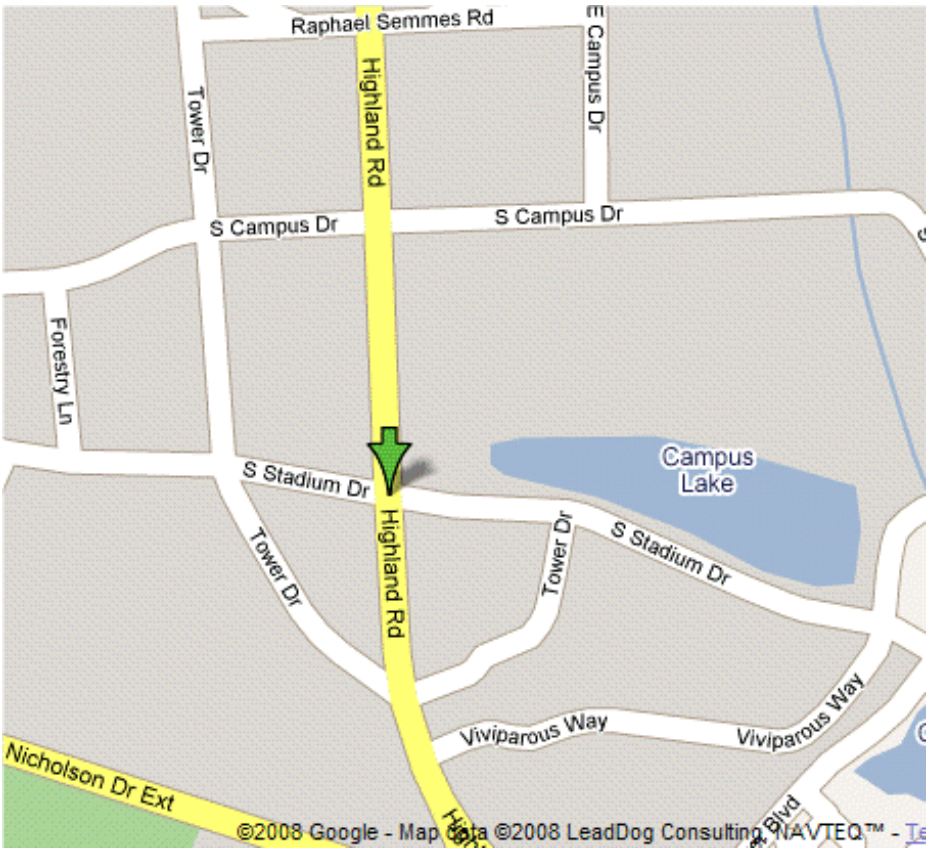
Appendix D.
Picture Gallery

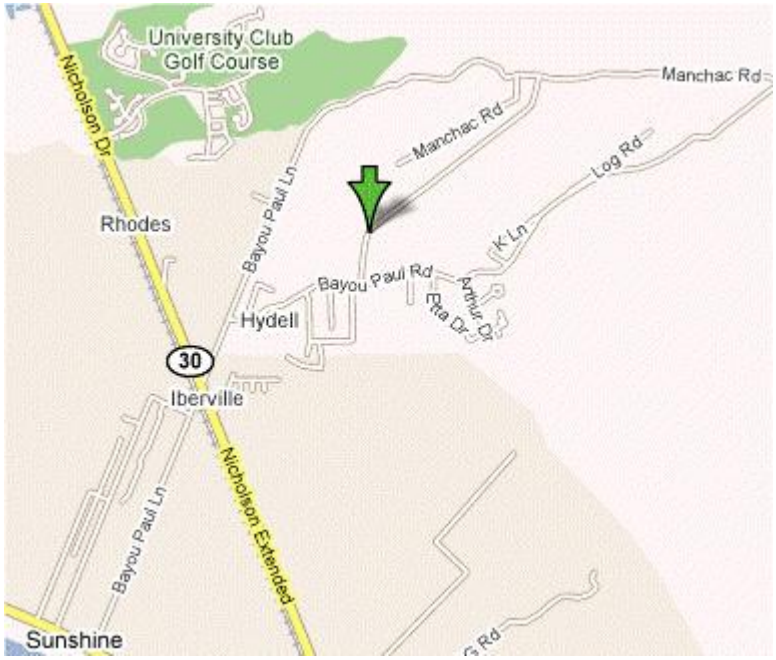
LSU Burden Center
Wood 1: Water Oak (*Quercus nigra*)



LSU Campus (Corner of S. Sadium Dr. and Highland Rd.)
Wood 4: Live Oak (*Quercus virginiana)*

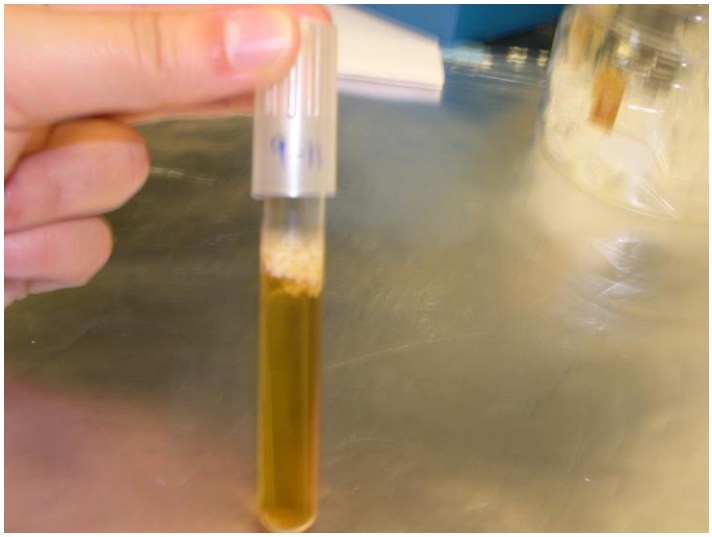


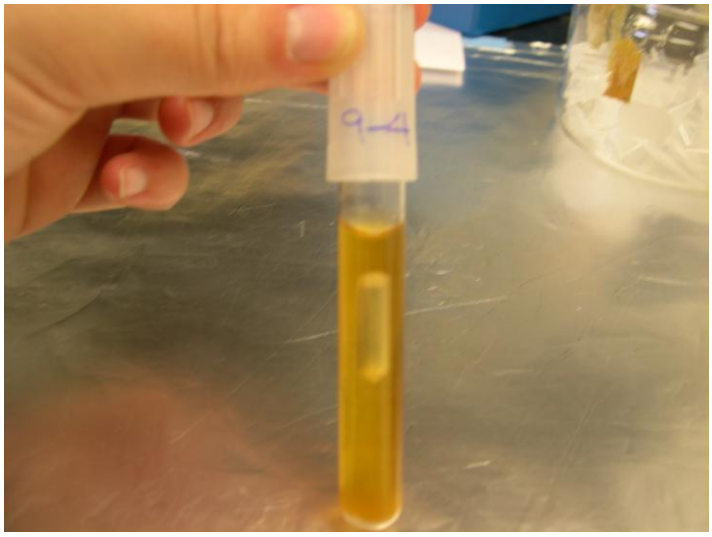
Dr. Larry Rouse's Home (Ascension Parish)
Wood 9: Pecan Wood (*Carya illinoinensis*)

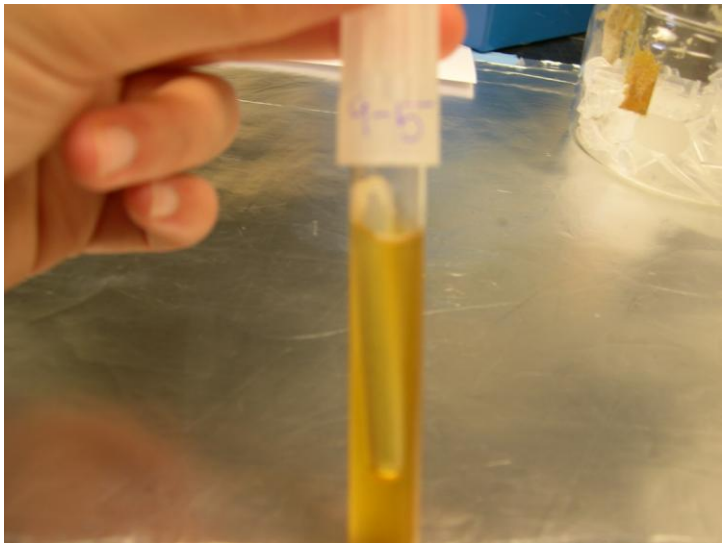



LSU

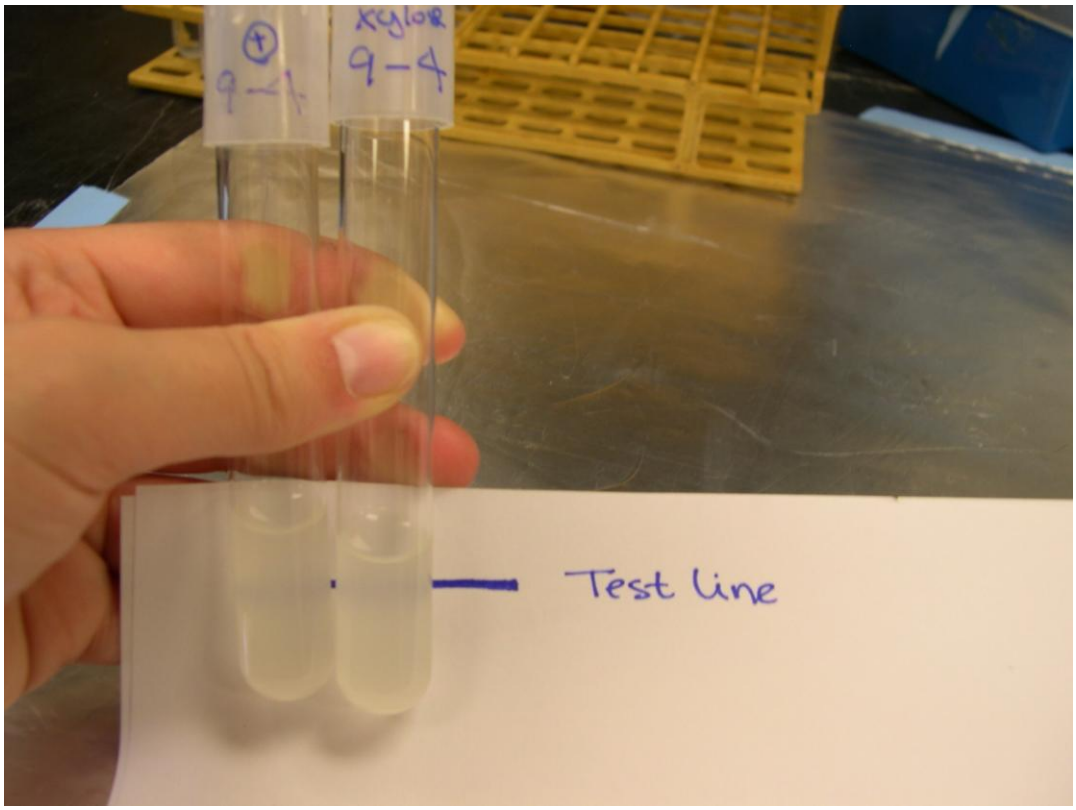
Xylose Fermentation Test







Xylose Assimilation Test



Lab (Appendix C) with the 10th and 11th Grade Biology Students at University High Lab School

April 15, 2008