AIMS Bioengineering

AIMS Bioengineering, 11(1): 1–7.
DOI: 10.3934/bioeng.2024003
Received: 25 September 2023
Revised: 25 December 2023
Accepted: 08 January 2024
Published: 29 January 2024

http://www.aimspress.com/journal/Bioengineering

Research article

Isolation, genetic identification of Amazonian yeasts and analysis of thermotolerance and alcohol tolerance of *Saccharomyces cerevisiae* from *Theobroma grandiflorum* and *Eugenia stipitate*

Flávia da Silva Fernandes[1,2], Luan Reis Honorato da Silva[2], Érica Simplício de Souza[3], Lívia Melo Carneiro[4], João Paulo Alves Silva[4], Steven Zelski[5], João Vicente Braga de Souza[2,*], Jacqueline da Silva Batista[6]

[1] Graduate Program in Genetics, Conservation and Evolutionary Biology - GCBEv, National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil
[2] Mycology Laboratory, National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil
[3] School of Technology, State University of Amazonas (UEA), Manaus, Amazonas, Brazil
[4] Department of Chemical Engineering, School of Engineering of Lorena, University of São Paulo (USP), Lorena, São Paulo, Brazil
[5] Miami University, Department of Biological Sciences, Middletown, OH, USA
[6] Thematic Laboratory of Molecular Biology, National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil

* **Correspondence:** Email: joao.souza@inpa.com; Tel: +5592991147815.

Abstract: Although yeasts of the *Saccharomyces cerevisiae* species are industrially significant, few studies have investigated their presence in environmental samples from the Amazon rainforest. This study aimed to isolate *S. cerevisiae* yeasts associated with trees of the Amazon Forest and investigate their thermotolerance, alcohol tolerance, and single nucleotide polymorphism (SNP) characteristics, along with those of regional strains from previous research and reference strains from the industry. We collected fruits, bark and decaying plant material from *Theobroma grandiflorum*, *Spondias mombin* L., *Mangifera indica* L., and *Eugenia stipitate*, and isolated yeasts using the culture media. To identify the yeasts, we conducted morphological and biochemical analyses, including sugar assimilation and fermentation, and sequencing analyses of the rDNA (ITS and LSU (D1 and D2)). We also performed fermentation tests to determine the optimum temperature, thermotolerance and ethanol tolerance. Finally, we subjected the selected strains to SNP analysis to study the reported genes that are important

for alcohol tolerance in *S. cerevisiae*: FPS1 (farnesyl diphosphate synthase1) and ASR1/YPR093 (alcohol sensitive RING/PHD finger1) genes. As a result, we isolated 53 yeasts, and 10 of which exhibited a sugar assimilation and fermentation profile that was similar to that of *S. cerevisiae*. These ten isolates were identified using sequencing of the ITS and LSU regions, which revealed the species to be *Wickerhamomyces anomalus* (n = 4), *Torulaspora pretoriensis* (n = 3), *Debaryomyces hansenni* (n = 1), and *Saccharomyces cerevisiae* (n = 2). Through the analysis of the ASR1 and FPS1 regions, we found an SNP at nucleotide 1552 A > G (FPS1), which was associated with ethanol tolerance under our experimental conditions. This work is significant because it is one of the first studies to focus specifically on the isolation of *S. cerevisiae* from samples in the Amazon region. Furthermore, the SNP analysis allowed us to differentiate isolates that showed greater tolerance to ethanol.

Keywords: Amazonian fruits; *Saccharomyces cerevisiae*; SNPs; ethanol tolerance; yeast; thermotolerance

Supplementary

Supplementary Table 1. Sequence codes of the strains from the present study deposited in the NCBI database.

Isolate code	Species	NCBI number (ITS)	NCBI number (D1/D2 – LSU)
CSR-252	*Saccharomyces cerevisiae*	OQ305528	OQ290941
CSR-402	*Saccharomyces cerevisiae*	OQ305815	OQ311345
ACY-251	*Torulaspora pretorienses*	OQ311336	OQ311347
ACY-252	*Torulaspora pretorienses*	OQ305840	OQ291312
ACY-401	*Torulaspora pretorienses*	OQ305814	OQ305602
ACR-251	*Wickerhamomyces anomalus*	OQ305826	OQ291319
ACR-252	*Wickerhamomyces anomalus*	OQ296992	OQ311344
CCY-251	*Wickerhamomyces anomalus*	OQ311340	OQ291310
ASY-254	*Wickerhamomyces anomalus*	OQ305824	OQ291311
ASY-404	*Debaromyces hansennii*	OQ305841	OQ291318

Supplementary Table 2. Analysis of environmental and industrial S. cerevisiae strains for maximum ethanol concentration for fermentation. The environmental strains used were CSR-252 and CSR-402 isolated from litter of Theobroma grandiflorum, and AR1, AR-3, AR-4, AR-9, AR-12 and AR-13 isolated from Eugenia stipitate fruit. The industrial strains used were CAT-1, PE-2, ANGEL and US-05.

Isolated yeasts	Origin of strains	Maximun concentration of ethanol that allows fermentation
CAT-2	Industrial	15%
PE-2	Industrial	15%
ANGEL	Industrial	15%
US-05	Industrial	15%
CSR 252	Litter of *T. grandiflorum*	10%
CSR 402	Litter of *T. grandiflorum*	10%
AR-1	*E. Stipitata* Fruit	15%
AR-3	*E. Stipitata* Fruit	15%
AR-4	*E. Stipitata* Fruit	15%
AR-9	*E. Stipitata* Fruit	15%
AR-12	*E. Stipitata* Fruit	15%
AR-13	*E. Stipitata* Fruit	15%

*Note: * The experiments presented an error of less than 10% and the numbers presented have statistical difference (95% confidence, ANOVA, T test).

Supplementary Table 3. Analysis of environmental and industrial S. cerevisiae strains for optimum and maximum temperatures for fermentation. The environmental strains used were CSR-252 and CSR-402 isolated from the litter of Theobroma grandiflorum, and AR1, AR-3, AR-4, AR-9, AR-12 and AR-13 isolated from Eugenia stipitate fruit. The industrial strains used were CAT-1, PE-2, ANGEL and US-05.

Isolated yeasts	Origin of strains	Optimum temperature for fermentation	Maximum temperature that allows fermentation
CAT-1	Industrial	30 °C	40 °C
PE-2	Industrial	30 °C	40 °C
ANGEL	Industrial	30 °C	40 °C
US-05	Industrial	30 °C	40 °C
CSR 252	*Litter of T. grandiflorum*	30 °C	40 °C
CSR 402	*Litter of T. grandiflorum*	30 °C	40 °C
AR-1	*Eugenia stipitate fruit*	30 °C	40 °C
AR-3	*Eugenia stipitate fruit*	30 °C	40 °C
AR-4	*Eugenia stipitate fruit*	30 °C	40 °C
AR-9	*Eugenia stipitate fruit*	30 °C	40 °C
AR-12	*Eugenia stipitate fruit*	30 °C	40 °C
AR-13	*Eugenia stipitate fruit*	30 °C	40 °C

*Note: * The experiments presented an error of less than 10% and that the numbers presented have statistical difference (95% confidence, ANOVA, T test).

Supplementary Table 4. Multiple sequence alignment and sequencing analysis files showing SNPs at positions 1188, 1347 and 1552 of the FPS1 gene in Saccharomyces cerevisiae strains tolerant to concentrations of 15% (more tolerant) and 10% (less tolerant) of ethanol in the medium. Alignment was performed using the MUSCLE program in the Geneious Prime® software.

Substitution in base	Amino acid	Substitution in amino acid	Type of mutation		Type of yeast				
					Industrial strains (4)	Isolated from *E. stipitate* (6)	Isolated from *T.grandi-florum* (2)	Reference Yeast S288C (NM_001181863)	*S. cerevisiae* (CP006402.1) (CP006387.1) (CP008077.1)
				Ethanol tolerance	+	+	-	-	*
c.1552 A>G	Isoleucine	p.Iso518Val	Missense mutation		G	G	-	-	G
c.255 A>G	Arginine	-	Silent mutation		G	G	-	-	G
c.594 C>T	Glycine	-	Silent mutation		T	T	-	-	T
c.1347 G>A	Treonine	-	Silent mutation		A	A	-	-	A
c.1188 A>G	Leucine	-	Silent mutation		G	G	-	-	G

The original sequence is based on that found in the Saccharomyces Genome Database (http://www.yeastgenome.org/).

*Note: *Strains were not tested for tolerance to ethanol.

Supplementary Table 5. Multiple sequence alignment and sequencing analysis files showing SNPs at positions 291, 378 and 690 of the ASR1 gene in in Saccharomyces cerevisiae strains tolerant to concentrations of 15% (more tolerant) and 10% (less tolerant) of ethanol in the medium. Alignment was performed using the MUSCLE program in the Geneious Prime® software.

Substitution in base	Amino acid	Substitution in amino acid	Type of yeast				
			Industrial strains (4)	Isolated from *E. stipitate* (6)	Isolated from *T. grandiflorum* (2)	Reference Yeast S288C (NM_001181863)	*S. cerevisiae* (CP020223.1) (CP006207.2) (CP006185.2)
		Ethanol tolerance	+	+	-	-	*
c.72G>C	Glycine	-	C	-	-	-	C
c.90C>T	Asparagine	-	T	-	-	-	T
c.102G>A	Glutamic acid	-	A	-	-	-	A
c.150A>G	Glutamic acid	-	G	-	-	-	G
c.213T>C	Phenyl-alanine	-	C	-	-	-	C
c.291C>T	Aspartatic acid	-	T	-	-	-	T
c.378T>C	Cysteine	-	C	-	-	-	C
c.690C>G	Aspartatic acid	-	G	-	-	-	G

The original sequence is based on that found in the Saccharomyces Genome Database (http://www.yeastgenome.org/).

*Note: *Strains were not tested for tolerance to ethanol.