

# Evaluation of the tolerance of acetic acid and 2-furaldehyde on the growth of *Pichia stipitis* and its respiratory deficient

B. Ortiz-Muñiz · J. Rasgado-Mellado ·  
J. Solis-Pacheco · C. Nolasco-Hipólito ·  
J. M. Domínguez-González · M. G. Aguilar-Uscanga

Received: 7 January 2014 / Accepted: 21 March 2014 / Published online: 5 April 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** The use of lignocellulosic residues for ethanol production is limited by toxic compounds in fermenting yeasts present in diluted acid hydrolysates like acetic acid and 2-furaldehyde. The respiratory deficient phenotype gives the cell the ability to resist several toxic compounds. So the aim of this work was to evaluate the tolerance to toxic compounds present in lignocellulosic hydrolysates like acetic acid and 2-furaldehyde in *Pichia stipitis* and its respiratory deficient strains. The respiratory deficient phenotype was induced by exposure to chemical agents such as acriflavine, acrylamide and rhodamine; 23 strains were obtained. The selection criterion was based on increasing specific ethanol yield (g ethanol g<sup>-1</sup> biomass) with acetic acid and furaldehyde tolerance. The screening showed that *P. stipitis* NRRL Y-7124 ACL 2-1RD (lacking cytochrome c), obtained using acrylamide, presented the highest specific ethanol production rate (1.82 g g<sup>-1</sup> h<sup>-1</sup>). Meanwhile, the ACF8-3RD strain showed

the highest acetic acid tolerance (7.80 g L<sup>-1</sup>) and the RHO2-3RD strain was able to tolerate up to 1.5 g L<sup>-1</sup> 2-furaldehyde with a growth and ethanol production inhibition of 23 and 22 %, respectively. The use of respiratory deficient yeast phenotype is a strategy for ethanol production improvement in a medium with toxic compounds such as hydrolysed sugarcane bagasse amongst others.

**Keywords** Respiratory deficient · *Pichia stipitis* · Ethanol production · 2-furaldehyde · Acetic acid tolerance

## Abbreviations

$Y_x/s$	Biomass yield (g biomass g <sup>-1</sup> substrate)
$Y_{et/s}$	Ethanol yield (g ethanol g <sup>-1</sup> substrate)
$Y_{et/x}$	Specific ethanol yield (g ethanol g <sup>-1</sup> biomass)
$V_p$	Ethanol specific production rate (g ethanol g <sup>-1</sup> biomass h <sup>-1</sup> )
$X$	Biomass production (g L <sup>-1</sup> )
$X_0$	Biomass production without inhibitor (g L <sup>-1</sup> )
$k$	Acetic acid sensitivity
$t$	Time (h)
$P$	Acetic acid (g L <sup>-1</sup> )
$P_c$	Acetic acid critic concentration (g L <sup>-1</sup> )
$n$	Exponential adjustment
$X_e$	Biomass production with 2-furaldehyde (g L <sup>-1</sup> )
$P_e$	Ethanol production with 2-furaldehyde (g L <sup>-1</sup> )
$P_0$	Ethanol production without 2-furaldehyde (g L <sup>-1</sup> )

B. Ortiz-Muñiz · J. Rasgado-Mellado ·  
M. G. Aguilar-Uscanga (✉)  
Instituto Tecnológico de Veracruz, Unidad de Investigación y  
Desarrollo en Alimentos (UNIDA), Av. Miguel A. de Quevedo  
2779, Col. Formando Hogar, C.P. 91860 Veracruz, Mexico  
e-mail: gaguilar@itver.edu.mx

J. Solis-Pacheco  
Centro Universitario de Ciencias Exactas e Ingeniería,  
Universidad de Guadalajara, Boulevard Marcelino García  
Barragán #1421, Col. Olímpica, C.P. 44430 Guadalajara,  
Jalisco, Mexico

C. Nolasco-Hipólito  
University of Malaysia Sarawak, Kuching, Malaysia

J. M. Domínguez-González  
Departamento de Ingeniería Química, Facultad de Ciencias,  
Universidad de Vigo, Campus Ourense, España. As. Lagoas s/n,  
32004 Ourense, Spain

## Introduction

Boris Ephrussi and colleagues in 1949 discovered respiratory deficient (RD) phenotype strains, also known as petite yeasts because the colonies are comparatively small [1]. Cytochromes a + a<sub>3</sub> and b had been damaged, so that the respiratory chain