Quantitative assessment of DNA damage in the industrial ethanol production strain *Saccharomyces cerevisiae* PE-2

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INTRODUCTION

The prospect of a limited fossil oil supply combined with environmental concerns associated with its use suggests the need for **alternative raw materials** for the production of fuels and chemicals.

Lignocellulosic raw materials such as wood residues and sugarcane bagasse are attractive as feedstocks as they are widely available, inexpensive and can be harvested in a sustainable way (Jansen et al. 2017). Lignocellulosic hydrolysates are also the most accessible and technically feasible substrates for **second generation biofuels and green chemicals**.

During the pretreatment process, the high temperatures and extremes of pH used lead to production of many by-products that are **fermentation inhibitors**, such as furans from the cellulose and hemicellulose fractions (Jönsson and Martin 2016).

Fermentation inhibitors can have an important effect on the **genetic stability** of the production strain, which can affect the attainable process efficiency and thereby also process economics.

The **yeast DEL assay** has been extensively used as an alternative to the Ames test for assessing the mutagenic properties of a wide range of compounds (Ku et al. 2007).

The standard DEL assay, established in the strain RS112, is based on **auxotrophic nutritional markers**, *HIS3* and *LEU2*, which are not useful in the industrial prototrophic and often polyploid strains used in industrial fermentation. We have therefore developed a novel version of the DEL assay, based on the loss of a dominant marker for geneticin resistance and the gain of a dominant marker for hygromycin B resistance.

OBJECTIVE

The **dominant DEL (dDEL) assay** was established in a laboratory strain CEN.PK and in the ethanol production strain *Saccharomyces cerevisiae* PE-2 and was successfully used to measure *in-vivo* DNA damage by hydrogen peroxide (H_2O_2) and furfural.

The dDEL assay should be a useful tool in the selection of new robust yeast strains and in designing hydrolysate production strategies and process setup.

METHODS

1) Plasmid and dDEL cassette assembly:

- Assembly of pPS1 carried out by *in-vivo* gap repair between seven linear fragments to form a circular vector;

- Amplification of the dDEL segment in three fragments that contained homologous terminal regions with each other and with the flanking sites of the *HIS3* locus.

Construction of the dominant DEL cassette (dDEL)

2) The yeast DEL and dDEL assays:

- H_2O_2 : cell cultures at $OD_{600}=1$ treated with 4 mM H_2O_2 for 0, 5, 10, and 20 min;

- Furfural: cell cultures at $OD_{600}=1$ treated with 0, 5, 10, 15 and 20 mM furfural for 17 h;

- Cells were spotted in rich or selective plates, and colonies were counted after 2 d of incubation at 30 °C.

Genomic rearrangement associated with activation of the yeast dDEL assay

RESULTS

Recovery time optimization

Comparison of dDEL frequencies and cell viabilities for the yeast dDEL assays with different recovery times using 4 mM H_2O_2

A proof of concept

DEL and dDEL recombination frequencies and cell viabilities for strains RS112, CEN.PK dDEL and PE dDEL treated with H_2O_2

Resistance to furfural

Recombination frequencies and cell viabilities for strains RS112 and PE dDEL respectively carrying the DEL or dDEL cassette

CONCLUSIONS

The ethanol production strain PE dDEL displayed **higher resistance** to the genotoxic insults caused by H_2O_2 and furfural than the laboratory strain CEN.PK.

Genetic stability has a large impact on fermentation processes and the dDEL assay should prove to be an important tool in the **screening of better performing industrial yeast strains and in process design**.

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