Generation of a Ustilago maydis ade2 mutant

Article · May 2016

3 authors:

Anju Verma
University of Missouri
24 PUBLICATIONS 180 CITATIONS
SEE PROFILE

Tamas Kapros
University of Missouri - Kansas City
22 PUBLICATIONS 328 CITATIONS
SEE PROFILE

Jakob H Waterborg
University of Missouri - Kansas City
78 PUBLICATIONS 1,681 CITATIONS
SEE PROFILE
Generation of a *Ustilago maydis* ade2 Mutant

Anju Verma1, Tamas Kapros2 and Jakob H Waterborg1*

1Division of Plant Sciences and Bond Llife Sciences Center, University of Missouri, Columbia, MO, USA
2Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO, USA
*Corresponding author: Jakob H Waterborg, Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO, USA, Tel: 1-913-219-5414; E-mail: Waterborg@umkc.edu

Received date: Apr 29, 2016; Accepted date: May 23, 2016; Published date: May 30, 2016

Abstract

The need exists to create the auxotrophic mutants for basidiomycete *Ustilago maydis* to allow selection of gene transformants in minimal growth media. The ADE2 gene was identified by homology with *Saccharomyces cerevisiae*. Adenine-requiring auxotrophic mutants were effectively created by homologous recombination in protoplasts using a standard plasmid containing the ADE2 locus interrupted by a phleomycin resistance gene, selected on standard complex media. The knockout ade2 mutant produced grows on minimal, defined medium only if supplemented with adenine.

Keywords: Ustilago; Selectable marker; Auxotrophy, Adenine

Introduction

Phytopathogenic *Ustilago maydis* is a haploid, dimorphic fungal model organism [1] that has been used to characterize genes involved in its infection of maize [2], in DNA recombination, mating determination and various signaling pathways [3,4]. We have used it to identify structural and regulatory genomic mutants of the two histone H3 variant loci using the fungicide carboxin as a selectable marker [5]. Carboxin has been used routinely in suspension cultures and in planta [6-8]. Other antibiotic selection systems have been used but these are not effective in minimal media [9]. In need for new selection alternatives to create additional gene transformants on minimal media in strains that already contained the carboxin-resistance insert, we turned to the auxotrophic mutant option.

Few auxotrophic mutants have been described for Ustilago, likely because they cannot be used for selection in planta. An uracil-requiring mutant [10] was created by disrupting the Pr4 gene [11]. The leu1 mutant [12] cannot be recreated in any desired background. Adenine auxotrophic mutants have been developed by knocking out a putative ADE6 in Ustilago strain 521 [13]. This result could not be reproduced in our laboratory.

Based on the published genomic sequence of Ustilago [4], we used BLAST searches to identify homologous loci for well-known mutants of *S. cerevisiae*. The 828 amino acid putative anthranilate synthase component II locus (XP_011388676) was identified as a homolog of the yeast TRP1 locus (Y DR007wp). However, in Ustilago, this tryptophan synthesis gene is fused to the TRP3 (Ykl211c) gene and thus was not pursued further. We identified homologous genes for the yeast histidine HIS3 loci YOR202w and YNL338w but were unable to obtain knockout transformants that were dependent on histidine in minimal media (results not shown), possibly due to differences in metabolic pathways between Ustilago and yeast. Yeast ADE2 is the required purine biosynthetic gene for phosphoribosylaminomimidazole carboxylase (AIR carboxylase). Knockout ade2 strains produce pink colonies on non-selective media [14]. Ustilago ade2 knockout strains, dependent on adenine supplementation of minimal media, were created using a single plasmid, PKO_Ade2_Phleo, available upon request. It consists of a phleomycin-resistance cassette that interrupts the Ustilago gene sequence XM_011389059.1, a homolog of yeast ADE2.

Materials and Methods

*Ustilago maydis* 521 (wild type strain 9021) was obtained from the Fungal Genetics Stock Center (UMKC, Kansas City, MO, USA). Cells were grown at 30°C in defined minimal medium containing 6.7 g Yeast Nitrogen Base without amino acids (Fisher Scientific, BD Difco, Germantown, MD, USA) and 20 g glucose in 1 L water or complex YEP5 medium containing 4 g yeast extract (BD Difco, Germantown, MD, USA), 4 g bactopeptone (BD Difco, Germantown, MD, USA) and 20 g sucrose in 1 L water with or without 20 g agar (Fisher Scientific). Phleomycin (Invivogen) and adenine (Sigma-Aldrich) were used at final concentrations of 30 mg/L and 10-25 mg/L, respectively. Phleomycin plates were stored at 4°C overnight before use. Plasmid DNA was isolated from E. coli using a Qiagen mini prep Kit (Qiagen, Germantown, MD, USA).

Infusion primers (Invitrogen, Grand Island, NY, USA) were designed using IDT Primer Quest software. Primer 1: Um_7z_Ade2_F: 5'-CGA AAT CGA TAA GCT GCT GAC GCC ACA TCG TTG AGA GG-3'. Primer 2: Um_Ade2_R: 5'-CTC ACT CAC GAC TCG AGC GAG AGA TTC TCA AA-3'. Primer 3: Um_Ade2_Phleo_F: 5'-CGA AAT CGA TAA GCT GCT GAC GCC ACA TCG TTG AGA GG-3'. Primer 4: Um_Phoeo_Ade2_R: 5'-CTC ACT CAC GAC GCC ACA TCG TTG AGA GG-3'. Primer 5: Um_Ade2_int_F: 5'-TGG CAG CAG GAG AGA TGG CCA CCA TGG CTT AGT GC-3'. Primer 6: Um_Ade2_7z_R: 5'-CTC ACT CAC GAC TCG AGC GAG AGA TTC TCA AA-3'. Primer 7: Um_Ade2_int_R: 5'-TGG CAG CAC GAG AGA TGG CCA CCA TGG CTT AGT GC-3'.

The phleomycin gene was obtained from pMF1-P, kindly provided by Michael Perlin (University of Louisville, Louisville, KY, USA). pGEM-7Zf(+) was used as the vector scaffold using an In-Fusion HD Cloning Kit (Clontech, Mountain View, CA, USA) to ligate the PCR fragments (Figure 1).
Results and Discussion

The yeast ADE2 gene (Open Reading frame YOR128C, 1716 bp, intron-free) on chromosome 15 codes for the 484 amino acid phosphoribosylaminoimidazole carboxylase. BLAST searching identified its homolog on chromosome 2 of the U. maydis genome [4] as the 1905 bp XM_011389059.1 locus. It codes for a putative 635 amino acid phosphoribosylaminoimidazole carboxylase. This locus was positively identified with an E-value of 9e-22 with 72% identity and 30% coverage for protein. The next best matched locus had an E-value of 0.40 and 1% coverage.

The In-Fusion method was selected to create a knockout cassette with at least 1 kb flanking sequences required for site-specific homologous recombination in Ustilago [15]. Primers were designed to produce PCR fragments that contained approximately 1 kb upstream of the transcription start site and 458 bp coding sequence followed by 682 bp downstream of the stop codon. The ble phleomycin resistance gene cassette was amplified by PCR from the pMF1p vector to replace the central coding region of the XM_011389059.1 locus (Figure 1). The three amplified fragments were ligated into the pGEM-7Zf(+) vector using an In-Fusion method and transformed into E. coli DH5α cells. Transformation of U. maydis protoplasts was performed using the plasmid named pKO-Ad2-Phleo as reported previously [5]. In short, 5 μL plasmid DNA (1 mg/mL) was used to transform 106 protoplasts using an In-Fusion method and transformed into E. coli DH5α cells.

Figure 1: Map of pKO_Ade2_Phleo plasmid obtained by insertion of the upstream and downstream sequences of Ustilago ADE2 and the ble phleomycin-resistance gene at the Hind III site of pGEM-7Zf(+).

This plasmid construct was transformed into E. coli DH5α cells and named pKO_Ade2_Phleo. Ustilago was transformed as described [5]. Genomic DNA was isolated from putative ade2 colonies by the glass bead technique described [5] and used to confirm homologous recombination by colony PCR, followed by amplification and sequencing of the insert.

Genomic DNA was isolated from putative ade2 colonies by the glass bead method described [5] and used to confirm homologous recombination by colony PCR, followed by amplification and sequencing of the insert.

Initially these colonies were pale pink, as seen in ade2 mutants in yeast and Candida albicans [14,17], but this accumulation of pink colour was lost during the repeated rounds of selection. This experimental result demonstrates a simple method to produce adenine-dependent auxotrophic mutants of Ustilago using plasmid pKO_Ade2_Phleo. Note that application of CRISPR-cas9 technology, recently described for Ustilago [18] with an efficiency of 70%, similar to that reported here, provides an alternative strategy to create ade2 knockout and other auxotrophic mutants.

Acknowledgement

This research was supported by the Missouri Life Sciences Research Board, award 13254 to JHW.

References


