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# Galactose metabolism and toxicity in *Ustilago maydis*

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ABSTRACT

In most organisms, galactose is metabolized via the Leloir pathway, which is conserved from bacteria to mammals. Utilization of galactose requires a close interplay of the metabolic enzymes, as misregulation or malfunction of individual components can lead to the accumulation of toxic intermediate compounds. For the phytopathogenic basidiomycete *Ustilago maydis*, galactose is toxic for wildtype strains, i.e. leads to growth repression despite the presence of favorable carbon sources as sucrose. The galactose sensitivity can be relieved by two independent modifications: (1) by disruption of Hxt1, which we identify as the major transporter for galactose, and (2) by a point mutation in the gene encoding the galactokinase Gal1, the first enzyme of the Leloir pathway. The mutation in *gal1(Y67F)* leads to reduced enzymatic activity of Gal1 and thus may limit the formation of putatively toxic galactose-1-phosphate. However, systematic deletions and double deletions of different genes involved in galactose metabolism point to a minor role of galactose-1-phosphate in galactose toxicity. Our results show that molecular triggers for galactose toxicity in *U. maydis* differ from yeast and mammals.

## 1. Introduction

In species ranging from bacteria to mammals, the metabolism of galactose to glucose-1-phosphate occurs through the highly conserved enzymes of the Leloir pathway. In this pathway, galactose is first phosphorylated to galactose-1-phosphate (gal-1P) by a galactokinase (Gal1p/GALK; the first name refers to the terminology used in *S. cerevisiae*, the second name refers to humans). Galactose-1-phosphate uridyl transferase (Gal7p/GALT) then transfers UMP from UDP-glucose to gal-1P, generating UDP-galactose and glucose-1-phosphate. UDP-galactose epimerase (Gal10p/GALE) subsequently converts UDP-galactose to UDP-glucose, the substrate for the transfer reaction of UMP to gal-1P. Additionally, phosphoglucomutase (PGM) converts glucose-1-phosphate to glucose-6-phosphate, the final product of the Leloir pathway that can enter different metabolic pathways as, e.g., glycolysis (Fig. 1, reviewed in Sellick et al., 2008). Filamentous fungi as *Aspergillus nidulans*, *Aspergillus niger* or *Trichoderma reesei* encompass a second, oxidoreductive pathway to metabolize galactose, starting in *T. reesei* with the xylose reductase XYL1, which converts galactose to galactitol (Fig. 1). Although individual reactions of the pathway differ between these fungi and some steps are still under discussion, it is likely that galactitol is metabolized via sorbitol and fructose to fructose-6-phosphate (Karaffa et al., 2013; Seiboth and Metz, 2011).

Several fungi have lost the capability to grow on galactose as carbon source due to the loss of single or all enzymes of the catabolic pathways (Slot and Rokas, 2010). One possible explanation for the loss of the pathway is that uptake of galactose bears the risk of toxic intermediate compounds (McGary et al., 2013). Indeed, galactose appears to be toxic for various organisms. Galactose is toxic when supplied exogenously to plants in concentrations as low as 1 mM (Loughman et al., 1988; Maretzki and Thom, 1978) and prevents growth of different plant tissues and in cell culture. In humans, impairment of Leloir pathway enzymes can lead to a galactose sensitivity known as galactosemia. Symptoms encompass cataract formation, mental retardation and premature ovarian failure; infants who are exposed to galactose, i.e. in the form of milk, potentially develop lethal symptoms (Coelho et al., 2017).

*S. cerevisiae* is often used as a model organism to study the pathophysiological mechanisms underlying galactose toxicity, which have remained largely unknown despite decades of study (Broomfield et al., 2011; Lai and Elsas, 2000; Leslie, 2003; Mehta et al., 1999; Mumma et al., 2008). As in humans, galactose toxicity in yeast is associated with an impairment of the Leloir pathway enzymes. While deletion of the gene for the galactokinase Gal1p only renders yeast cells unable to utilize galactose as a carbon source, growth of Δ*gal7* (uridyl transferase)

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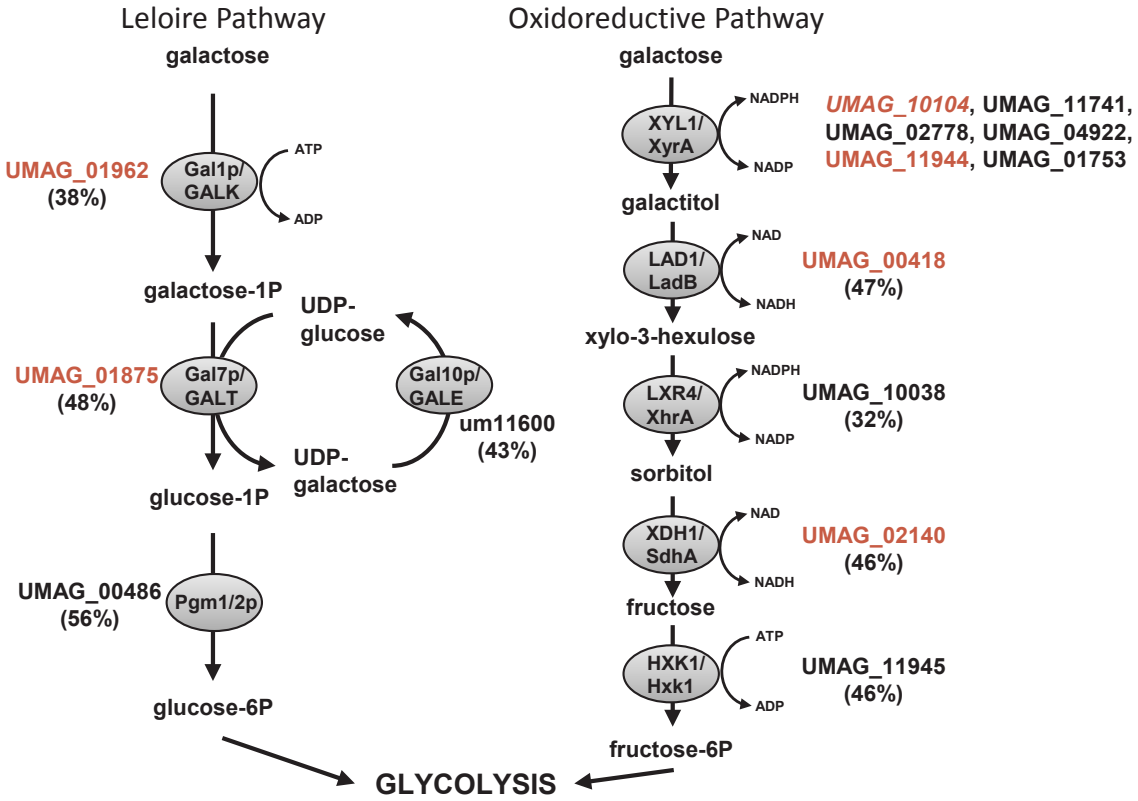


**Fig. 1.** Schematic illustration of the conserved galactose utilization Leloir and oxidoreductive pathways. Abbreviations (for Leloir pathway: *S. cerevisiae/human;* for oxidoreductive pathway: *Trichoderma reesei/Aspergillus niger*): Gal1p/GALK: galactokinase; Gal7p/GALT: galactose-1-phosphate uridyl transferase; Gal10p/GALE: UDP-galactose epimerase; Pgm1/2p: glucose-1-phosphatephosphoglugomutase; XYL1/XyrA: xylose reductase; LAD1: arabitol dehydrogenase; LadB: galactitol dehydrogenase; LXR4/XhrA: xylo-3-hexulose reductase; XDH1/SdhA: xylitol dehydrogenase; HXK1: hexokinase. Systematic protein names for *U. maydis* refer to the annotation of the NCBI Gene Database; % values indicate amino acid sequence identities of *U. maydis* proteins to homologous proteins in *S. cerevisiae* or *Trichoderma reesei.* Accession numbers and sequence identities are given in Table S3.

or Δ*gal10* (epimerase) cells is completely arrested already at low concentrations of galactose, even in the presence of an alternative carbon source as ethanol or glycerol. Δ*gal7* or Δ*gal10* cells accumulate unusual high concentrations of gal-1P. Galactose sensitivity in Δ*gal7* cells can be relieved either by loss of the galactokinase gene *GAL1* or by overexpression of the gene for UDP-glucose pyrophosphorylase Ugp1p, decreasing the cellular gal-1P level. Thus, gal-1P is believed to play a major role in galactose toxicity (Douglas and Hawthorne, 1964; Lai and Elsas, 2000; Mehta et al., 1999), possibly through inhibition of important enzymes such as phosphoglucomutase, inositol monophosphatase or UDP-glucose pyrophosphorylase (Gitzelmann, 1995; Lai and Elsas, 2000; Lai et al., 2003; Mehta et al., 1999; Sidbury, 1960). More recent studies assessing the correlation of galactose metabolite concentrations and galactose-induced growth arrest suggest that gal-1P accumulation is not solely responsible for the toxic effects of galactose. In addition, galactitol and, in case of GALE-deficiency, UDP-galactose seems to play additional roles (de Jongh et al., 2008; Mumma et al., 2008).

In this study we have investigated the toxic effect of galactose in the phytopathogenic basidiomycete *Ustilago maydis*. *U. maydis* is the causative agent of corn smut disease. As a biotrophic organism, this fungus relies on its host plant *Zea mays* for completion of its life cycle. In contrast to most other biotrophic fungi, *U. maydis* can easily be grown in axenic culture and is amenable to a broad range of molecular biological techniques (for review see Brefort et al., 2009; Vollmeister et al., 2012).

Similar to plants, galactose prevents growth of *U. maydis* even in the presence of alternative carbon sources as sucrose. The observed galactose sensitivity is largely abolished after deletion of *hxt1*, encoding a high affinity hexose transporter required for glucose-mediated growth in axenic culture (Schuler et al., 2015). We show that Hxt1, in addition to its function in glucose transport and glucose sensing, also resembles the major transporter for galactose uptake in *U. maydis*. Similar to the *hxt1* deletion, increased galactose tolerance results from a mutation in *gal1* that leads to reduced galactokinase activity. Apparently, *U. maydis* can metabolize galactose when Gal1-P concentrations in the cell are low. Surprisingly, galactose sensitivity is not released by deletion of *gal1*, as described for *S. cerevisiae*, suggesting that gal-1P is not the sole toxic intermediate for *U. maydis*.

## 2. Materials and methods

### 2.1. Strains and growth conditions

*Escherichia coli* strain TOP10 (Invitrogen) was used for cloning purposes. *U. maydis* strains used in this study are listed in Table S1. Routinely, *U. maydis* cells were grown in Yeast Extract Peptone Sucrose Light (YEPSL) liquid medium (Brachmann et al., 2001). For RNA extraction, *U. maydis* was grown in glutamine array (GA) medium, which is based on the minimal medium described by Holliday (Holliday, 1974) with 30 mM L-glutamine as nitrogen source. *S. cerevisiae* strain BY4717Δ*gal1* (MATa deletion library, Open Biosystems) was used for analyses of Gal1(Y67F) activity. BY4717Δ*gal1* was grown in minimal medium (0.67% yeast nitrogen base supplemented with the required amino acids) containing 2% glucose at 29 °C. Complementation studies in BY4717Δ*gal1* were carried out on minimal medium containing the indicated hexose concentrations.