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The lack of *UME6* sustain 2- deoxy-D-glucose toxicity under inositol limitation condition in *Saccharomyces cerevisiae*

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Abstract- The glucose analogues 2-DG exposure affects glycolytic enzymes. Glucose-6 P is the precursor for the synthesis of *myo*-inositol metabolism, 2-DG exposer reduced *myo*-inositol level. In the current study, we found *UME6* deletion depict resistance to the 2-DG toxicity. The *UME6* deletion increased the *INO1* β-galactosidase activity even the exposer of 2-DG in (0.025% and 0.05%) under I− condition compared to WT cells. The sever growth impairment was observed in 0.025% concentration of 2-DG, and also severally affects the *INO1* transcription in WT cells under inositol deprivation. The 0.05% concentration highly toxic no growth was observed under inositol limitation, which led to abolish the *INO1* transcription in WT cells. The *UME6* transcription factor negatively regulates *INO1* transcription this could be the reason for the resistance *ume6*Δ strain of 2-DG toxicity with I− condition. We conclude that the *ume6*Δ resistant with the exposure of 2-DG under inositol limitation condition in *Saccharomyces cerevisiae*.

Keywords- 2-deoxy-D-glucose, *INO1*, β-Galactosidase activity, over production of inositol.

I. INTRODUCTION

Yeast *UME6* gene encode unscheduled meiotic gene expression which represses transcription of a diverse set of genes involved in meiosis (1), heat shock response and arginine catabolism (1,2). The Ume6p also a positive regulator of some early meiotic genes in sporulating cells (1, 2). However, Ume6p also positively regulates the CDP-DAG pathway genes *CHO1*, *CHO2* and *OPI3* but negatively regulates *INO1* as well as the phospholipid biosynthetic genes in yeast (2).

Glucose-6-P is the precursor for carbohydrate storage metabolism (trehalose, glycogen) and also inositol metabolism (4).The *INO1* encode inositol-3-P synthase catalyzed the conversion of Glucoe-6 P to inositol-3 P (3). Previous report suggest that the 2-deoxy-D-glucose (2-DG) is a well-known glucose analogue and the exposure leads to defective glycolysis (5). The 2-DG exposure causes multiple defects growth impairment, cell morphology, cell wall biosynthesis, and cell lysis (4). Glucose-6 P procure from glucose with the help of (*HXK*).

The hexokinase also catalyzed the conversion of glucose analogue 2-DG to 2-DG-6 P, that toxic substance cannot metabolized (6, 7).Inositol is an essential metabolites modulates a diverse cellular process including apoptosis (8), membrane trafficking (9), and are involved in glycolipid and phospholipid metabolism as well (10). Hence, inositol level also defect under 2-DG exposure in mice (7).

The deletion of *UME6* displays over production of inositol (Opi− phenotype) and negatively regulates *INO1* transcription (2). The *INO1* transcription was positively regulated by Ino2-Ino4 heterodimer complex, Opi1p repress the *INO1* under inositol presence condition (8,9). Similarly, previous study also depict deletion of *OPI1* rescue the sensitivity of 2-DG under inositol deprivation condition (10). The deletion of *GCR1* also depict highly sensitive with the exposure of 2-DG under normal condition (10).

In this work we established the importance of meiotic regulating transcription factor *ume6*Δ under 2-DG sensitivity in *Saccharomyces cerevisiae*. Additionally, we examine the *INO1-LacZ* activity to exposure of glucose analogue (2-DG) in WT and *ume6*Δ either presence or absence of inositol and 2-DG (0.025% and 0.05%). The current study depict the *ume6*Δ (Opi− phenotype) strains rescue the 2-DG toxicity under inositol deprivation condition.

II. MATERIALS AND METHODS

Media, strains and growth condition.

The yeast wild type (BY4741: *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and *ume6*Δ strains were gifted by Prof. Ram Rajasekharan, Central Food Technological Research

Institute (CFTRI), Mysore, India. Yeast strains was grown in YPD (1% in yeast extract, 2% peptone, and 2% dextrose) medium (pH 7.0) or synthetic complete/ defined media contains yeast nitrogenous base 0.67g (YNB), yeast dropout with respective amino acids (0.14g uracil 7.6 mg, tryptophan 7.6 mg, leucine 38 mg, and histidine 7.6 mg) and 100 mg/L kanamycin with aeration at 30 °C at 180 rpm for 24 h. The cell growth was monitored by measuring the absorbance at 600nm. The YEp357R vector containing *INO1-LacZ* was gifted from John M. Lopes, College of Natural Sciences, University of Massachusetts, Amherst, MA. The yeast *INO1-LacZ* containing YEp357R vector was used for promoter reporter analysis. Cells were grown until mid-log phase in 5 mL of SD-U containing 2% glucose. Then the cells were pelleted and resuspend with fresh SD-U medium containing presence (I+) or absence (I-) of inositol cells were continue shaking with 180 rpm at 30°C. The *E. coli* transformants was selected on LBA plates containing 0.5% yeast extract, 1% peptone, 1% NaCl, and 100 mg/L ampicillin.

Cell tolerance assessment of 2-Deoxyglucose (2-DG) resistance by plate assay and growth curve analysis.

The overnight culture of WT and *ume6*Δ cells were grown in YPD media at 30 °C. The cells were equalized by OD_{600} and equal number of cells were serially diluted (10-fold) with autoclaved double distilled water, and 3 µl aliquots of each dilution were spotted onto SC and the media containing either presence or absence of inositol with and without exposure of 0.025% and 0.050% of 2-DG and 2% dextrose as a carbon source containing agar plates and incubated 30 °C for 3 days. The growth curve analysis cell growth was monitored by measuring the absorbance ($A_{600\ nm}$) of the cell density at indicated times points until 64 h.

β-Galactosidase activity of INO-LacZ

Wild-type and *ume6*Δ strains were transformed with YEp357- *INO1-LacZ* fusion gene containing plasmid and the transformants were grown in to SD-U (synthetic defined uracil) media up to mid-log phase at 30 °C. Then the cells were washed with fresh SD-U media and cells were subcultures in to SD-U containing either I- or I+, and the presence or absence of 0.025% and 0.050% of 2-DG and incubated at 30 °C for 4 h. The cell free extract were collected and extracted protein was quantified by Bradford method [13] and measured the β-Galactosidase activity as described previous method of Rose and Botstein [14]. The *INO1-LacZ* activity was expressed nmol. min^{-1}. mg^{-1} protein.

Statistical analysis

The experimental data quantification was examined by using Student's t-test, and the obtained variance were considered statistically significant *p<0.05. Respectively at least three independent experiments was repeated. Data are presented as the average ± standard deviation (SD). Statistical examination was performed using the sigma plot 10.0 software.

III. RESULTS AND DISCUSSION

Growth assessment and *INO1- LacZ* activity in WT and *ume6*Δ strain under both presence and absence of inositol.

In the present study, we examine the growth using spot assay and growth curve analysis in WT and *ume6*Δ strain under inositol limitation (I−) and inositol supplementation. We found an increases the growth Inositol is an important for the cell growth (10), the lack of inositol reduces the growth in WT cells (Fig. 1B).

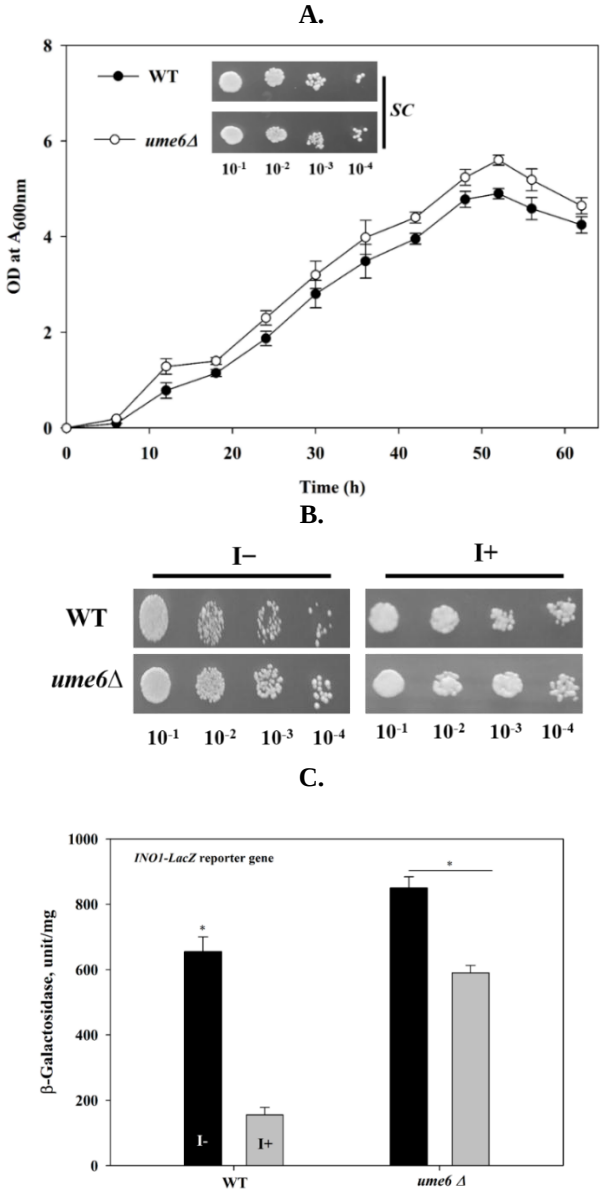


Fig.1 Effect of growth under presence and absence of inositol and *INO1*- LacZ activity in WT and *ume6*Δ.

The WT and *ume6*Δ strain were cultured in YPD media and grown up to mid-log phase at 30 °C. (A). The growth curve was monitored by measuring OD (A_{600nm}) of the cells at indicate time points until 64 h. The growth curve is an average of three experimental repeats. The cells were serially diluted (tenfold), and 3 µl of cells were spotted onto SC 2% dextrose containing agar plates and incubated for 3 days at 30 °C (Insert image spot test). (B). The mid log phase grown cells were serially diluted, and 3µl of cells were spotted onto SC 2% dextrose with the presence or absence of inositol containing agar plates and incubated for 3 days at 30 °C. (C). β-galactosidase activity. The cell harbouring YEp357R plasmid containing *INO1-LacZ* fusion gene containing WT cells were cultured in SD-Ura. The mid-log phase grown cells were washed with I− free media and cells were seeded into both presence and absence of inositol. The cell extract were subjected to measure the β-Galactosidase activity. The specific β-Galactosidase activity was expressed as units mg-1 (nmol min-1 mg-1). The data shown is average of three independent experiments (*P < 0.05).

The *UME6* is a negative regulator of *INO1* transcription (2), hence the deletion of *UME6* also shown to increases the growth under I− (Fig. 1B). Normally, *INO1* highly expressed under I− condition (8,9). The *INO1* promoter fused with *LacZ* gene also increased (~28%) under I− and in *ume6*Δ compared to WT I− cells. The activity of β-galactosidase still increased under I+ in *ume6*Δ (Fig. 1C), which indicates the deletion of *UME6* increased the growth under I− condition compared to WT cells.

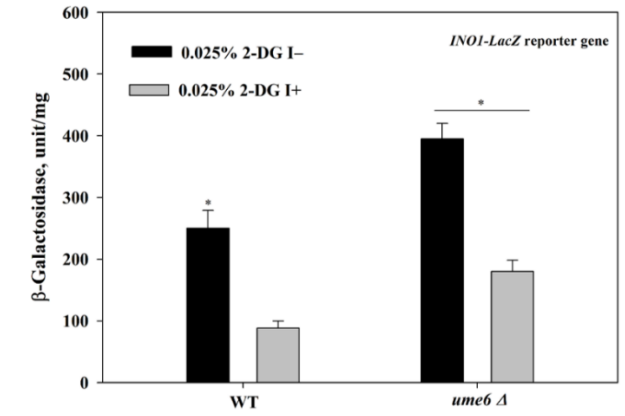
Effect of 0.025% 2-DG on *INO1-LacZ* activity in the presence and absence of inositol.

The lack of Ume6p display Opi−phenotype and increased the *INO1*-LacZ expression (Fig.1). Further we established the β-galactosidase activity of *INO1* under I− and I+ with the exposure of 0.025% 2-DG. Because the presence of 2-DG leads to increases a toxic substance 2-DG phosphate, which cause on inhibitory effect on *myo*-inositol-1-phosphate production (11). The deletion of *UME6* cells with the 0.025% 2-DG exposure increased the activity of *INO1-LacZ* under I− and I+ when compared to WT cells (Fig.2 A), but reduced the expression of *INO1-LacZ* compared to untreated *ume6*Δ cells (Fig.1C).

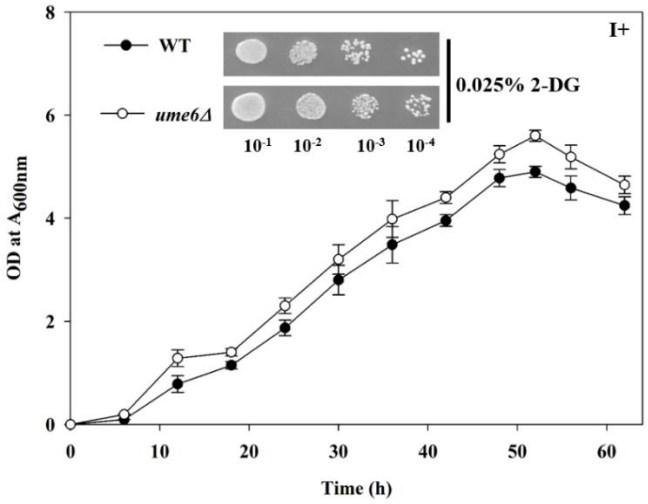
Growth assessment of 0.025% 2-DG on plate assay and growth curve analysis under I− and I+.

Further we examined 0,025% 2-DG sensitivity using plate assay and growth curve analysis. We found reduced growth (growth curve and spot assay) was observed under I+ condition with the exposure of 0.025% 2-DG exposure in WT cells (Fig. 2B). The *ume6*Δ still increased the growth was observed under I+ condition compared to the respective WT cells (Fig.2B).

A.



B



C

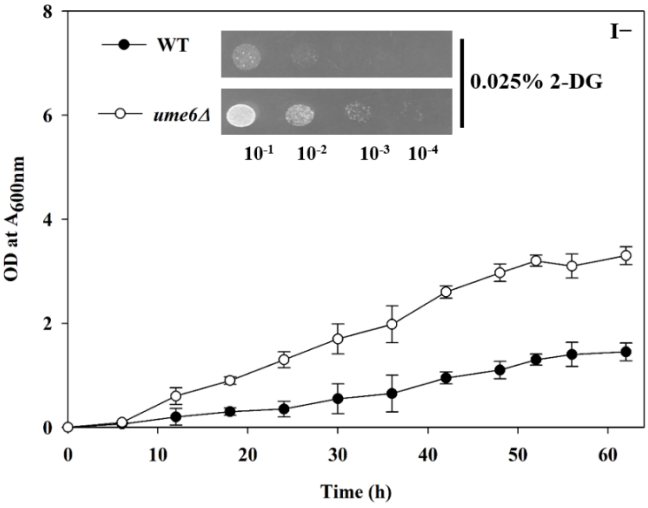


Fig.2 β-Galactosidase activity of *INO1-LacZ* and growth assessment under 0.025% of 2-DG in WT and *ume6*Δ cells

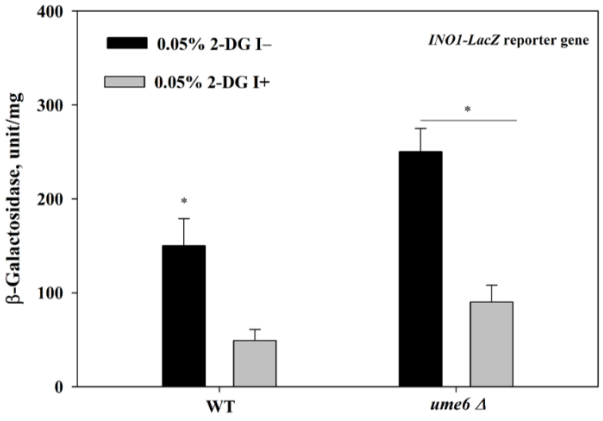
under I− and I+. (A). WT and *ume6*Δ strains harbouring YEp357R plasmid containing *INO1-LacZ* fusion gene and transformants were cultured into SD-U media up to mid log phase and the cells were washed and seeded in to the fresh media with the presence of 0.025% 2-DG under I− and I+ cells. The cell extract were subjected to measure the β-Galactosidase activity. The specific β-Galactosidase activity was expressed as units mg-1 (nmol min-1 mg-1). (B and C) Spot test and growth curve analysis of WT and *ume6*Δ strains under the exposure of 2-DG (0.025%) in the presence and absence of inositol. The data shown is average of three independent experiments (*P < 0.05).

The growth resistivity was supported for the increased β-galactosidase activity of *INO1-LacZ* in *ume6*Δ cells under I+ condition compared to WT cells (Fig. 2B). Similarly, the deletion of *OPI1* (Opi− phenotype) also rescue the growth with the exposure of 2-DG toxicity (10). Further, inositol limitation condition drastic growth reduction was observed in the exposure of 0.025% of 2-DG exposure (Fig. 2C).

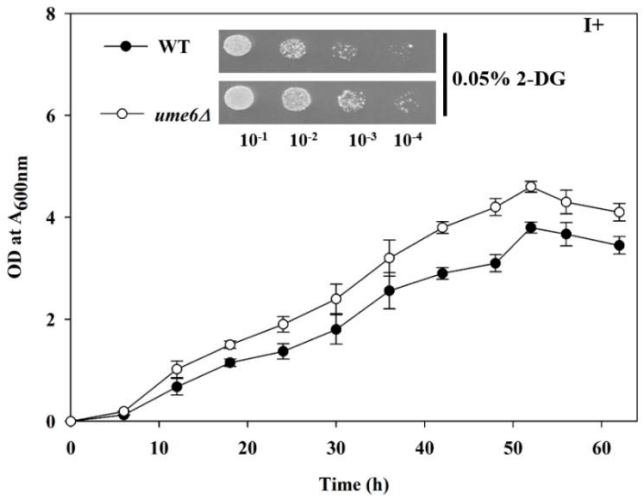
Effect of 0.025% 2-DG on *INO1-LacZ* activity and toxicity assessment on plate assay and growth curve analysis under I− and I+.

The *INO1* promoter activity and growth was measured in WT and *ume6*Δ cells during the 2-DG (0.05%) exposure under I− and I+ condition. The presence of inositol the exposure of 0.05% 2-DG exposure observed a stringed growth in WT cells (Fig. 3B) compared to WT 0.025% 2-DG exposure (Fig.2 B). Further, the drastic reduction of *INO1-LacZ* activity was observed under I− condition, which indicates the increased concentration 2-DG highly toxic to the cells in WT cells under I− (Fig. 3A and C).

A.



B.



C.

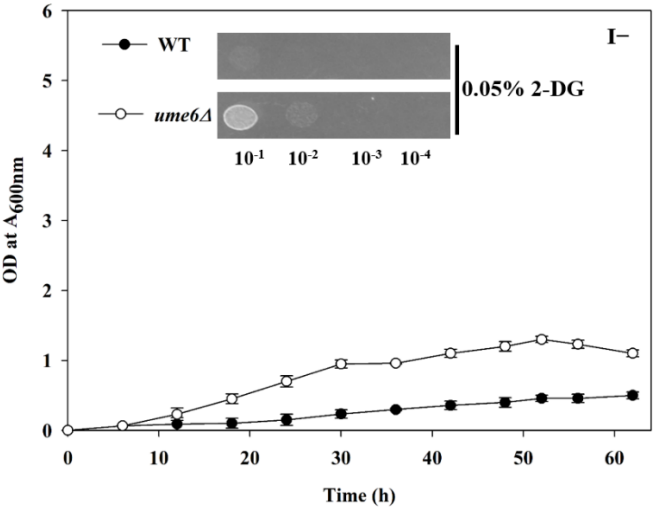


Fig.3. Effect of 0.05% 2-DG in β-Galactosidase activity of *INO1-LacZ* and growth assessment of WT and *ume6*Δ cells under I− and I+. (A).

WT and *ume6*Δ strains harbouring YEp357R plasmid containing *INO1-LacZ* fusion gene and transformants were cultured into SD-U media up to mid log phase and the cells were washed and seeded in to the fresh media with the presence of 0.025% 2-DG under I− and I+ cells. The cell extract were subjected to measure the β-Galactosidase activity. The specific β-Galactosidase activity was expressed as units mg-1 (nmol min-1 mg-1). (B) and (C). Spot test and growth curve analysis of WT and *ume6*Δ strains under the exposure of 2-DG (0.05%) in the presence and absence of inositol. The data shown is average of three independent experiments (*P < 0.05).

On the other hand the inositol supplementation also reduced growth was observed in WT cell with the exposure of 0.05%

2-DG. The deletion of *UME6* still observed the growth in the exposure of 0.05% 2-DG under I− but not in WT cells, which depict the absence of inositol rescued the growth with the presence of 0.05% 2-DG (Fig. 3C). Finally the deletion of *UME6* sustained the growth under I− with the presence of glucose analogue 2-DG toxicity.

IV. CONCLUSION.

The exposure of glucose analogue 2-DG causes to reduces the growth and *INO1-LacZ* activity. Inositol was required for the growth under 2-DG toxicity. Hence the Ume6p negatively regulates the *INO1* transcription, even the deletion of *UME6* increased the *INO1* transcription. The increased expression of *INO1* leads to rescued the growth under I− condition with the exposure of 2-DG (0.025% and 0.05%) in *ume6Δ* cells. Our study we conclude that the decreased *INO1-LacZ* leads to impaired the growth in the presence of 2-DG under I−. The inositol supplementation resistant to the 2-DG toxicity. Further work is need to find out the role of *UME6* in 2-DG toxicity under inositol limitation condition.

V. ACKNOWLEDGMENT

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