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Research Paper

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In *Saccharomyces cerevisiae* the attenuation of the 2- deoxy-D-glucose toxicity is alleviated by inositol

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Abstract: In *Saccharomysis cerevasiae* the glycolytic transcription factor *GCR1* tightly controls the expression of genes involved in glycolysis but the importance of Gcr1 transcription in 2-DG toxicity is still elusive. In the present study, we observed 2-DG toxicity in the wild-type, *gcr1*Δ, and *ino2*Δ cells under inositol absence (I−) condition obstruct the growth, hence the *opi1*Δ cells were resistant to 2-DG exposure. Further, the presence of 2-DG significantly decreased the promoter activity of *INO1* in the WT cells. The 2-DG exposure suppressed the growth rate in WT and *gcr1*Δ strains under inositol limitation condition, but the *opi1*Δ (over production of inositol) strain was resistant to the 2-DG exposure with I− condition. Taken together, the above findings suggest that 2-DG exposure reduced the promoter activity of *INO1* leads to inositol auxotrophic growth defect, hence the inositol supplementation bring back normal. To conclude that the inositol alleviates the 2-DG toxicity in *Saccharomyces cerevisiae.*

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

I. INTRODUCTION

The transcription factor *GCR1* (Glycolytic regulation 1) is an important regulator of glycolytic gene expression in yeast [3] and glycolysis is the primary metabolic pathway used rapidly by yeast for cell proliferation and to ferment glucose to ethanol. In yeast 2-deoxy-D-glucose (2-DG) is an established glucose analogue and the carbohydrate metabolism is altered and leads to defective glycolysis [1, 2]. The 2 DG inhibits growth, affects cell morphology, cell wall biosynthesis and cell lysis [1].

In glycolysis, *HXK* (hexokinase) activates glucose to glucose-6-phosphate (G-6P), which is used up in the HMP shunt pathway or other metabolic routes including inositol pathway [6, 7]. The hexokinase also phosphorylates 2-DG to 2-DG-6-phosphate (a toxic substance) that cannot be further metabolized [4, 5]. In the 2-DG treated mice, there is a significant reduction in *myo*-inositol level. In *Saccharomyces cerevisiae* the inositol derivatives modulate diverse cellular functions such as apoptosis [12] membrane trafficking [11] and are involved in phospholipid and glycolipid metabolism as well.

The inositol-3-phosphate synthase (*INO1*) synthesizes inositol-3-phosphate from glucose-6-phosphate [11]. The exposure of 2-DG accumulates 2-DG-6-phosphate and inhibits the formation of *myo*-inositol phosphate due to the inhibitory effect of 1-L-*myo*-inositol 1-phosphate synthase [8]. The transcription of *INO1* was positively controlled by Ino2-Ino4 heterodimer complex, whereas Opi1 is a negative regulator. During the excess availability of inositol, the Opi1p is translocated from ER to nucleus where it interacts with *INO2* transcription factor leading to the repression of *INO1* transcription [10, 13].

The 2-DG has been extensively studied and has been revealed to obstruct the metabolic events in various eukaryotic species. In the present study, we examined the importance of exogenous inositol supplementation for alleviating the effect of glucose analogue (2-DG). Additional, we established the importance of *INO1* promoter activity under various 2-DG concentration in wild-type cells and the behaviour of *OPI1* deletion under inositol limitation condition. The current research revealed that the exogenous inositol improves the 2-DG toxicity in yeast.

II. MATERIALS AND METHODS

Media and growth condition.

The yeast wild-type (BY4741: *MATa his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*) and strains *gcr1*Δ, *opi1*Δ, and *ino2*Δ 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 (SC) / defined (SD) media containing yeast nitrogenous base 0.67 g (YNB), yeast dropout with respective amino acids (0.14 g 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 600 nm. 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 studies. Cells were grown until mid-log phase in 5 mL of synthetic defined uracil (SD-Ura) containing 2% glucose. The cells were pelleted and resuspended with fresh SD-Ura medium in the presence (I+) or absence (I−) of inositol (75 µM) and the cells were continued 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 resistance by spot test assays.

The overnight culture of WT, *gcr1*Δ, *opi1*Δ and *ino2*Δ cells were grown in YPD media at 30 °C. The cells were normalized by OD ($A_{600\ nm}$) 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.

β-Galactosidase activity of INO-LacZ assay

The wild-type strains were transformed with YEp357-*INO1-LacZ* fusion gene containing plasmid and single colony transformants were grown in synthetic defined uracil (SD-Ura) media up to mid-log phase at 30 °C. The cells were then washed with fresh SD-Ura media and sub-cultured in SD-Ura media with or without inositol (I− or I+), and in the presence or absence of 2-DG (0.025% and 0.050%) and incubated at 30 °C for 4 h. The cell free extract was collected, protein extracted and quantified by Bradford method [19]. The β-Galactosidase activity was measured as described previously by Rose and Botstein [20]. The *INO1-LacZ* activity was expressed as nmol. min^{-1}. mg^{-1} protein.

Statistical analysis

The experimental data quantification was analyzed using Student's t-test, and the obtained difference was considered statistically significant *$p<0.05$. At least three independent experiments were repeated andata are presented as the average ± standard deviation (SD). Statistical analysis was performed using the sigma plot 10.0 software.

III. RESULTS AND DISCUSSION

Effect of 2- DG on the growth of WT, *gcr1*Δ, *opi1*Δ, and *ino2*Δ.

Exposure of cells to 2-DG (highly toxic compound) resulted in growth inhibition, and this might be due to the altered metabolism mainly the glycolysis and HMP shunt pathway (16). In the present study, we examined the effect of 2-DG (glucose analogue) during the deletion of transcription factors in glucose (*gcr1*) and lipid metabolism (*opi1*Δ, *ino2*Δ). To study the growth, we used spot assay using the synthetic completed media contains 2% glucose (SC-D)) in the presence and absence of 0.025% and 0.050% DG. The exposure of 0.05% 2-DG caused a significant inhibition with cell growth in WT, *gcr1*Δ, *ino2*Δ cells, but still the growth of *opi1*Δ cells slightly increased compared to WT cells (Fig. 1). Previous results revealed that over expression of *GCR1* suppressed the 2-DG toxicity mildly in glucose containing media [18]. The *gcr1*Δ cells were highly sensitive to 0.05% 2-DG (Fig.1) and confirmed that 2-DG inhibited the yeast cell growth under fermentation condition.

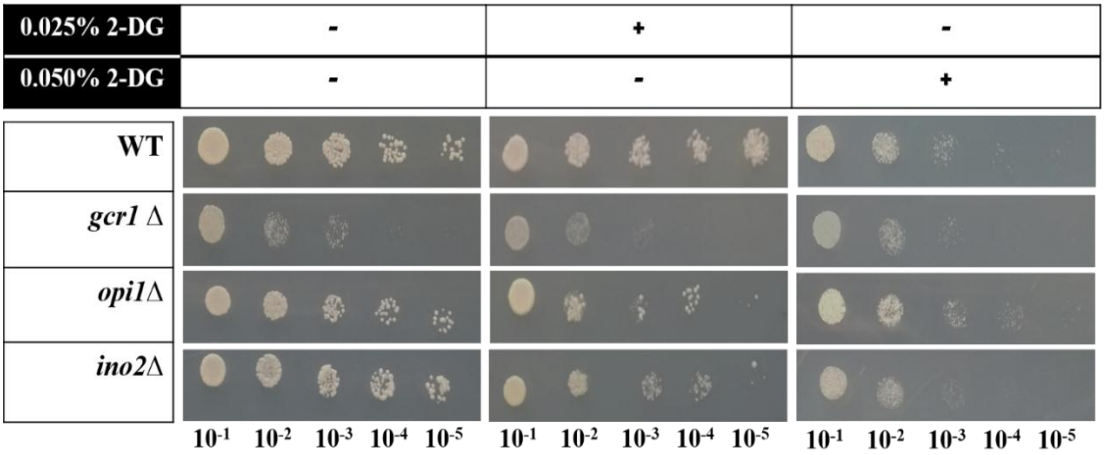

Fig.1. Cell tolerance assessment of 2-DG in control and deletion strains

The WT and deletion strains were cultured in YPD media and grown up to mid-log phase at 30 °C. The cells were serially diluted (10- fold), and 3 µl of cells were spotted onto SC 2% dextrose agar plates in the presence or absence of 2-DG (0.025% and 0.050%) and incubated for 3 days at 30 °C.

Effect of 2-DG in *INO1 lacZ* activity in WT cells.

The presence of 2-DG accumulates 2-DG-phosphate (toxic) which causes an inhibitory effect on *myo*-inositol-1-phosphate formation [8]. In yeast the inositol-3-phosphate synthase (*INO1*) synthesizes inositol-3-phosphate from glucose-6-phosphate [11]. We analyzed the promoter activity of *INO1* gene using β-galactosidase assay. In the wild-type cells with 0.025% and 0.050% 2-DG exposure ~35% and ~60% reduction were observed respectively when compared to the control (WT untreated) cells (Fig.2). We performed the promoter reporter assay of *INO1-LacZ* activity and the data confirms that 2-DG is a toxic to the cells and affected the *INO1* β-galactosidase activity.

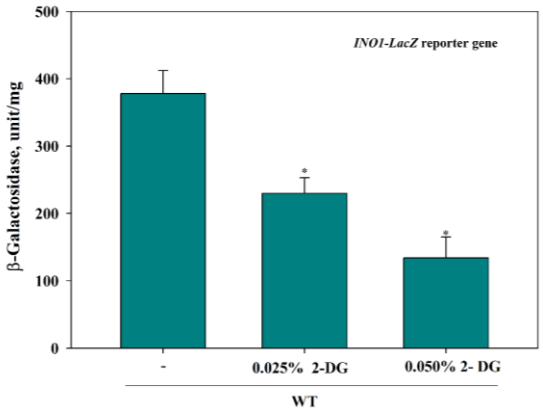

Fig.2. β-Galactosidase activity of *INO1-LacZ* in WT cells under 2- DG exposure

The WT cells harbouring YEp357R plasmid containing *INO1-LacZ* fusion gene was cultured in SD-Ura media up to mid log phase with or without 2-DG (0.025% and 0.050%) and the β-Galactosidase activity was measured in the cell extract. The β-Galactosidase specific activity was expressed as units mg-1 (nmol min-1 mg-1) incomplete. The data shows the average of three independent experiments (*P < 0.05).

Effect of 2- DG on β-Galactosidase activity of *INO1-LacZ* in WT cells in the presence or absence of inositol.

We also performed the promoter reporter assay of *INO1-LacZ* activity in different combination of 2-DG in the presence (I+) or absence (I−) of inositol. The *INO1* expression is highly expressed under inositol limitation condition [10, 11], and we also found increased *INO1-LacZ* activity under I− condition in WT cells compared to I+ (Fig.3). The β-Galactosidase activity of WT cells was substantially lower with the presence of 0.025% 2-DG and was further reduced with 0.050% 2-DG in the WT cells with inositol presence when compared to WT cells under I− condition (Fig.3) and the comparable reduction was observed in WT I+ during 0.02% and 0.05% 2-DG exposure also (Fig.3). These results suggest that the inositol auxotrophy condition with the WT cells were highly sensitive to the exposure of 2-DG and reduced the *INO1* transcription in yeast cells.

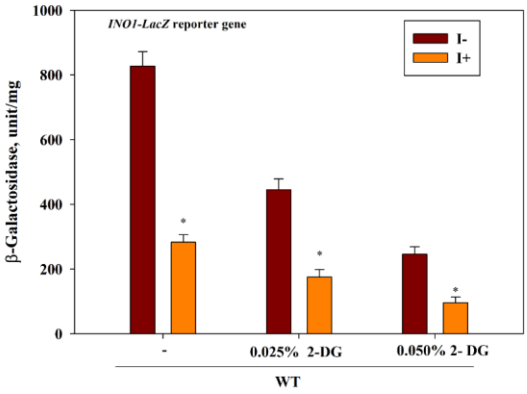

Fig.3. β-Galactosidase activity of *INO1-LacZ* in WT cells

The WT cells containing *INO1-LacZ* fusion gene transformants were allowed to grow up to mid-log phase in SD-Ura media at 30 ºC. The cells were then collected and washed with fresh SD-Ura I− media and resuspended with fresh SD-Ura I− and SD-Ura I+ media containing 0.025% and 0.050% 2-DG and allowed to grow for 4 h. The cells were collected and the β-Galactosidase activity measured in the cell extract. 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).

Spot test analysis of WT, *gcr1*Δ, *opi1*Δ, and *ino2*Δ cells in the presence or absence of inositol under exposure of 2-DG

The *OPI1* is a negative regulator of lipid metabolism, hence the *opi1*Δ cells showed an increase in growth under I− condition compared to WT cells [10]. Normally the lack Opi1 (indicator of over production of inositol), increases the growth under inositol limitation compared to control cells (Fig.4). The growth pattern was measured in the WT and deletion strains during the 2-DG exposure under I− and I+ condition. The *gcr1*Δ highly repressed the growth with 0.025% and 0.050% 2-DG under inositol limitation condition. The 2 DG exposure reduced the growth and was retrieved with inositol (75 µM) compared to WT cells without inositol (Fig.4). Normally the lack of *INO2* requires exogenous inositol for growth, and the deletion displays an inositol auxotrophy [10]. As a result, the growth was fully abolished with *ino2*Δ cells under 2-DG exposure with I− condition compared to I+.

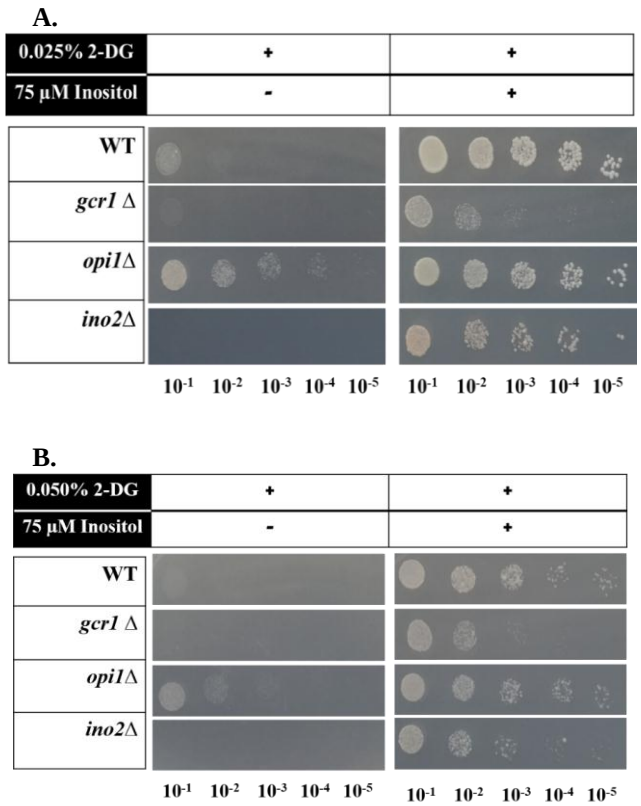

Fig.4. Growth study

The WT, *gcr1*Δ, *opi1*Δ, and *ino2*Δ cells were cultured in YPD media and grown up to mid-log phase at 30 °C. The cells were then serially diluted (10- fold), and 3 µl of cells were spotted onto agar plates with SC I− and SC I+ containing 2% dextrose with the presence or absence of 2-DG (0.025% and 0.050%) and incubated for 3 days at 30 °C.

The growth of the *opi1*Δ cells was more in the presence of 2-DG (0.025% or 0.050%) with I− condition compared to WT I− cells (Fig.4), implying the cells were resistant to 2-DG and growth was not affected in *opi1*Δ cells compared to the WT cells under I− condition.

IV. CONCLUSION.

The WT yeast cells were highly sensitive to the toxic compound 2 DG and the growth inhibited in I− condition. However, the growth was not inhibited with *opi1*Δ cells and was able to tolerate the 2 DG toxicity (0.025% and 0.050%) in I− condition. Our study, we conclude that the inositol is essential to alleviate the 2-DG toxicity in *Saccharomyces cerevisiae*. Further work is needed to study the molecular mechanism and the phenotypic importance of *OPI1* in 2-DG toxicity under inositol auxotrophic condition.

V. ACKNOWLEDGMENT

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