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*Candida albicans*에서 fructose-1,6-bisphosphate aldolase의 역할

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指導敎授 姜 思 旭

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副委員長 姜思旭 (인)

委 員 HYUN AH KIM (인)

ABSTRACT

Fructose-1,6-bisphosphate aldolase is one of the important glycolytic enzyme which catalyzes the reversible conversion of a fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate required for both glycolysis and gluconeogenesis. Herein, the gene encoding fructose-1,6-bisphosphate aldolase (*FBA1*) has been cloned from *Candida albicans*. The *FBA1* gene contained an open reading frame consisted of 1,080 bp that encodes a polypeptide consisting of 360 amino acids with a calculated molecular mass of 39,215 Da. To investigate the functional role of FBA1 in *C. albicans*, the *FBA1* gene was disrupted or overexpressed. The *fba1/fba1* null mutants were not viable in both rich and minimally defined medium. The *FBA1/fab1* heterozygous mutant exhibited the significant decreased RNA profile of *FBA1* contrast to the wild-type cells (*FBA1/FBA1*), significantly. However, the phenotypes in terms of the differentiation in *FBA1/fab1* heterozygous mutant displayed no difference compared to wild-type cells. However, the *FBA1*-overexpressing cells led to the morphological transition into true hyphae in glucose-containing rich medium, and true hyphal-specific gene *HWP1* was remarkably expressed through the northern blot analysis. Additionally, the intracellular methylglyoxal and reactive oxygen species concentrations were increased while the glutathione level was decreased inversely proportional to those of *FBA1*-overexpressing cells. This result suggests that the *FBA1* overexpression of *C. albicans* might lead to methylglyoxal accumulation in cells, significantly. Moreover, *FBA1*-overpexpressing cells underwent to G1-phase arrest of the cell cycle due to the methylglyoxal accumulation, which led to the formation of true hyphae. Therefore, *Candida albicans* fructose-1,6-bisphosphate aldolase overexpression caused to methylglyoxal accumulation leading to altered cell growth, differentiation and the cell cycle.

Key Words: *Candida albicans*, fructose-1,6-bisphosphate aldolase, methylglyoxal, differentiation

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LIST OF ABBREVIATIONS

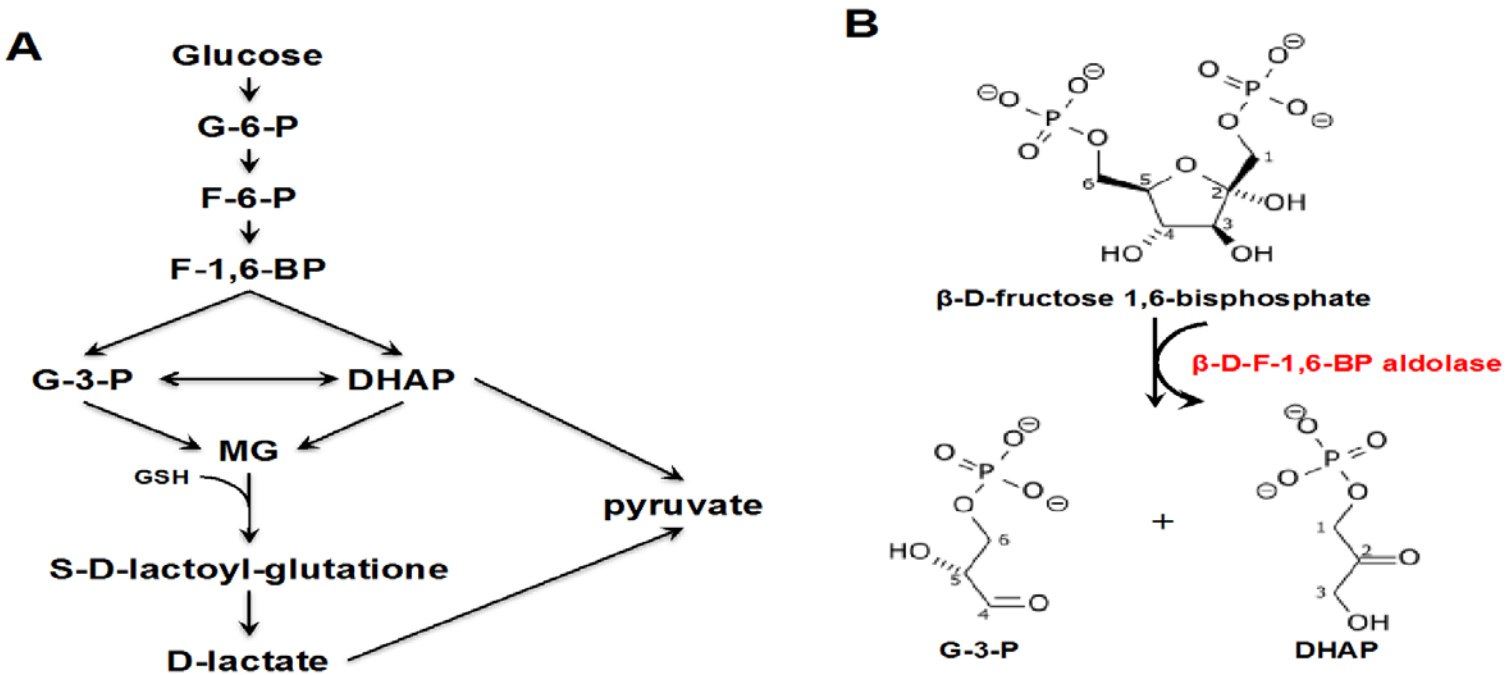
FBA1	fructose-1,6-bisphosphate aldolase
MG	methylglyoxal
GSH	reduced glutathione
HPLC	high performance (pressure) liquid chromatography
FOA	5-fluoroorotic acid
LiAc	lithium acetate
ROS	reactive oxygen species
DCFH	dichlorofluorescin
DCFH-DA	dichloroflurescin diacetate
PI	propidium iodide
PBS	phosphatase based saline
FACS	flow cytometry
OD	optical density
bp	base pair
nt	nucleotide
Da	dalton
PCR	polymerase chain reaction
ORF	open reading frame
U	unit

I. INTRODUCTION

Glycolysis is an essential for living species, both aerobes and anaerobes. It is a crucial pathway of glucose degradation and can generate energy without oxygen. To simplify, glucose ($C_6H_{12}O_6$) converts into pyruvates ($CH_3COCOOH$) and releases high-energy, adenosine triphosphate. In all major groups of microorganism, glycolysis can be found both prokaryotes and eukaryotes. Without the pathway, microorganisms have to metabolize an energy cost-ineffective way (Calderone, 2002; Odds, 1988).

Fructose-1,6-bisphosphate aldolase (Fba1p) is one of glycolytic enzymes which cleaves fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. So far, it has been studied that in *Saccharomyces cerevisiae*, the defective Fba1p allele lacked aldolase enzymatic activity and failed to grow on media containing glucose (Schwelberger *et al*., 1989; Lobo, 1984). In *Streptococcus bovis*, overexpression of Fba1p leads to increase in the ratio of formate to lactate production (Asanuma *et al*., 2004). In *Candida albicans*, the conditional mutant of Fba1p lost an enzymatic activity (Rodaki *et al*., 2006).

From glyceraldehyde-3-phosphate or dihydroxyacetone phosphate, methylglyoxal is produced by non-enzymatic elimination (Chan *et al*., 2007; Brownlee, 2005; Gome *et al*., 2006). Methylglyoxal (CH_3COCHO, MG) was thought to be the important regulating factor of glucose catabolism resulting in lactate formation (Bernhauer *et al*., 1929). MG is considered to be a toxic compound that causes DNA and protein damage both *in vivo* and *in vitro* in mammalian cells (Thornalley, 2008; Ramasamy, 2006). The biogenic amines with MG results in biologically active free radicals (Lee *et al*., 1998) and advanced glycation end-products (Szent-Györgyi and McLaughlin, 1975; Van Herreweghe *et al*., 2002). MG also triggers the activation of the stress-activated protein kinase cascade by acting as a signal molecule via a response regulator in the fission yeast *Schizosaccharomyces pombe* (Takatsume *et al*., 2006). Furthermore, MG production strongly correlates with the generation of superoxide radicals and



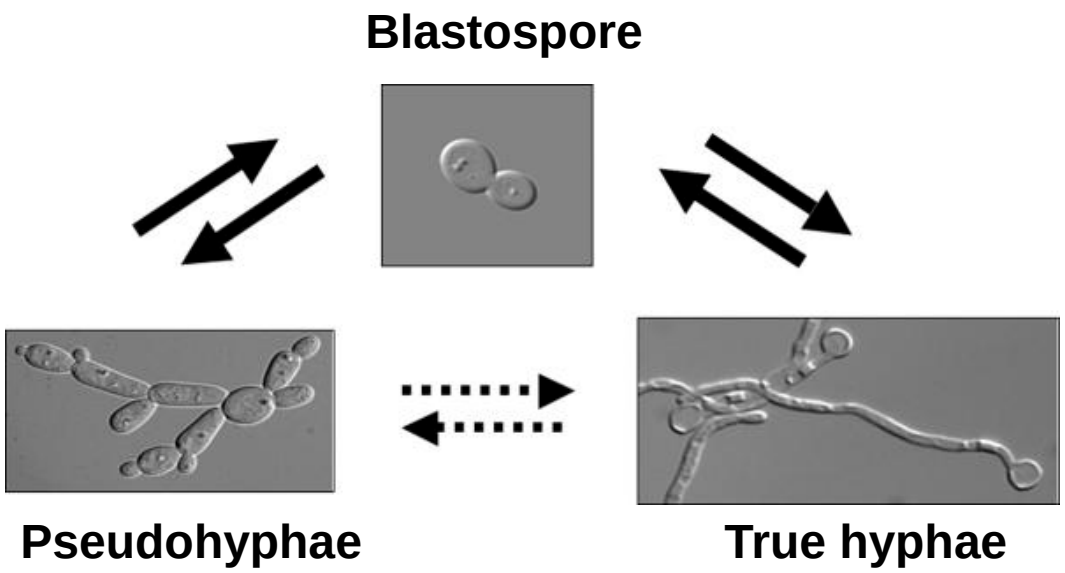
Scheme 1. Overall characteristics of methylglyoxal metabolism in cells.

(A) Partial pathway of methylglyoxal metabolism. G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; G-3-P, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone-phosphate; MG, methylglyoxal; GSH, glutathione. (B) Chemical structures involving in aldolase reaction (Kalapos, 1999).

reactive oxygen species during cell growth (Maeta *et al*., 2005; Du *et al*., 2001). Methylglyoxal metabolism has been little studied so far. Recently, methylglyoxal dehydrogenases, which catalyzes MG oxidation to pyruvate, has been reported in *C. albicans* (Kwak *et al*., 2014)

Candida albicans is a diploid opportunistic pathogenic fungus. This commensal fungus can cause serious disease in immunocompromised patients such as HIV and cancer patients who are in chemotherapy, when the normal flora in the oral and gastrointestinal tracts is disrupted. (Berman and Sudbery, 2002; Culter *et al*., 1991). Additionally, *C. albicans* has diverse cell morphogenesis from blastospore form to filamentous form including a thread-like hyphal growth form, germ tubes, and pseudohyphae. Morphology can be reversible depends on environmental conditions including pH, temperature and media. This feature is necessary for *C. albicans* to survive within a host. In general, yeast form predominates during mucosal colonization in a normal host but when a host's immune system defects, cells emerge into hyphae forms (Berman and Sudbery, 2002; Brown *et al*., 1999; Odds *et al*., 1985).

In spite of the potential importance of fructose-1,6-bisphosphate aldolase its roles in many biological events, its physiological phenomenon in *C. albicans* have been not known yet compared to other systems such as mammals. This study will present how fructose-1,6-bisphosphate aldolase affects *C. albicans* by molecular cloning , overexpression of the gene encoding fructose-1,6-bisphosphate aldolase. Furthermore, overexpression of fructose-1,6-bisphosphate aldolase may related to regulation of cell cycle and differentiation especially in response to methylglyoxal concentration which would be helpful to investigate in various intracellular phenomenon. This will give us insights of the roles of fructose-1,6-bisphosphate aldolase in *C. albicans*.



Scheme 2. Morphological transtions of *Candida albicans*.

Vegetative morphology of *C. albicans* cells. Yeast cells (top center) can form both pseudohyphae (lower left) and true hyphae (lower right). Switching between the pseudohyphal and hyphal morphologies is less frequent (Berman, 2006).

II. Materials And Methods

1. Materials

1.1 Yeast strains and growth conditions

The *C. albicans* strains used in this study are listed in Table 1. For the routine growth of *Candida* cells containing disrupted genes, cells were cultured in YPD (1% yeast extract, 2% peptone, 2% glucose) , minimally defined SD (2% glucose, 0.5% ammonium sulfate, 0.17% yeast nitrogen base without amino acids and ammonium sulfate) or Spider (1% nutrient broth, 1% mannitol, 0.2% K_2HPO_4) in liquid broth or 1.8% agar-containing plates as described previously [28]. Ura^- auxotrophs were selected on minimal defined medium supplemented with 625 mg 5-fluoroorotic acid and 25 mg uridine per liter (FOA medium). Prior to selection, cells were plated on YPD medium and incubated for 48 hours. Individual colonies were taken from the plate and suspended in sterile distilled water. About ~5×10^6 cells of suspension was spreaded FOA medium and incubated for 2-3 days. Before growing cells in liquid medium, stock cultures were grown on agar plates and stored at 4 °C. All cells were grown at 28 °C.

1.2 Bacterial strain and culture condition

Escherichia coli DH5α was used for DNA manipulation. *E.coli* strains were grown 37 °C in Lurea-Bertani (LB, 1% tryptone, 0.5% yeast extract, 1% NaCl) medium supplemented, where required, with the following antibiotic at final concentrations: ampicillin, 50 μg/ml.

Table 1. Bacterial and *Candida albicans* strains used in this study.

Strain	Genotype	Sources or reference
Bacterial strain		
E. coli DH5α	F-*ΔlacU169(Φ80lacZΔM15)endAlreclhsdR17 deoR supE44 thi-1 λ-gyrA96 relA1*	Hanahan, 1983
Candida albicans strains		
SC5314	Wild type isolate	Fonzi and Irwin, 1993
CAI4	Δ*ura*3::*imm*434/Δ*ura*3::434	Fonzi and Irwin, 1993
KU101	Δ*ura*3::*imm*434/Δ*ura*3::434 Δ*fba1*::*hph*-URA3-*hph*/*FBA1*	This work
KU102	Δ*ura*3::*imm*434/Δ*ura*3::434 Δ*fba1*::*hph*/*FBA1*	This work
KU103	Δ*ura*3::*imm*434/Δ*ura*3::434 (pYPB1-ADHpt)	This work
KU104	Δ*ura*3::*imm*434/Δ*ura*3::434 *FBA1*::*ADH1p*	This work

2. Methods

2.1. Lithium acetate transformation of *C. albicans*

To introduce recombinant construct to disrupt or overexpress, lithium acetate transformation was performed using URA auxotroph, *C. albicans* CAI4. The *C. albicans* cells were grown overnight from a portion of a single colony in 25mL YPD at 28 °C with agitation to a density of 1×10^7 CFU/ml (O.D. > 0.5) and cooled cells on ice and harvested by centrifugation at 9,000 rpm for 5 min at 4 °C. Harvested cells were washed twice with 10mM Tris-HCl, 1.0 mM EDTA buffer (TE buffer, pH 7.5), washed with TE/LiAc buffer (100 mM LiAc, 10 mM Tris-HCl; pH 7.5)and then resuspended pellet (5×10^8 cells) in 100 μl TE/LiAc buffer. Each transformation was added sequentially; 300 μl freshly prepared polyethylene glycol 3550 (PEG 3550, Sigma Aldrich), 50 μl of cells suspension, 60 μg carrier DNA (Clontech) and several μl recombinant DNA as prepared. Transforming cells were incubated for 30 min at 28 °C following heat shock at 42 °C for 15 min. After centrifugation 9,000 rpm for 15 s, supernatant were removed and resuspended pellet spread aliquots on selective medium and incubated at 28 °C for 2 to 3 days.

2.2. Genetic manipulation methods

General techniques for isolation and manipulation of DNA in E. coli were as previously described (Sambrokk and Gething, 1989). pGEM-T easy vector (Promega) was used for cloning of PCR product. Integrating expression vector pQF18 (Feng *et al*., 1999) and extrachromosomal expression vector YPB1-ADHPt (Csank *et al*., 1998) were used for introducing appropriate genes into *C. albicans*. The constructs and plasmids used in this study were summarized in Table 2.

2.3. Polymerase chain reaction (PCR)

DNA fragment amplification was performed according to the method recommended by Taq polymerase manufacturer (Promega, Madison, WI) with slight modification. For the reactions, 100 pmol of degenerated oligonucleotide primers, 200 ng of genomic DNA or 10 ng plasmid DNA and 0.5 units of Taq polymerases were combined in a final volume of 50 μl with reaction buffer (50 mM KCl, 1.2 mM MgCl2, 10 mM Tris-HCl, pH 8.4, 0.01% gelatin) containing 50 μM dNTPs. The reaction mixture was subjected to 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C and 1 min extension at 72 °C.

2.4. Disruption of *C. albicans FBA1*

FBA1 disruption was carried out as previously described by Fonzi and Irwin (1993) with modifications. The plasmids and primers used in this study are described in Table 2, 3. The 3.5-kb *Bam*HI fragment containing a *hph-URA3-hph* gene disruption cassette was isolated from pQF86 (Feng *et al*., 1999). Multi cloning sites of the original pUC18 backbone plasmid were used for cloning with a 3.5-kb *Bam*HI fragment containing a *Candida* gene disruption cassette. This fragment was ligated with *Bam*HI, yielding pQF181 and its reverse oriented pQF182, which were constructed as plasmids containing a gene disruption cassette that can be excised with *Sac*I/*Hin*dIII (Hwang *et al*, 2003). The resulting plasmids pQF181 and pQF182 were used for yeast transformation into the Ura⁻ homozygous strains CAI4 for efficiency. The 200-bp *Sac*I/*Kpn*I and 448-bp *Sal*I/*Hin*dIII digested fragments from pQF181-SK and pQF181-SH, that is, the flanking regions of upstream and downstream of the *FBA1* ORF, were consecutively inserted into the *Sac*I/*Kpn*I and *Sal*I/*Hin*dIII sites of *hph-URA3-hph* from pQF181 or pQF182 to remove the 1,080 bp *FBA1* coding region of *C. albicans* (Hwang *et al*., 2003). The resulting plasmid, pQF18-SH1, was linearized with *Sac*I/*Hin*dIII, and approximately 4.5 kb of the resulting fragments was used for transformation into CAI4, the Ura⁻ derivatives of SC5314, respectively. Spontaneous Ura⁻ strains from heterozygous KU102

disruptant was selected on SD plates supplemented with FOA to pop out the *URA3* locus by uracil prototrophy (Fonzi and Irwin, 1993).

2.5. *C. albicans FBA1* overexpression

To overexpress *FBA1* in *C. albicans*, a YPB1-ADHPt vector containing the *C. albicans ADH1* promoter and terminator regions was used (Talibi *et al*., 1999). PCR amplification was performed by using primers including a *Bgl*II site for the forward and a *Xho*I site for the reverse fragments containing the *FBA1* ORF (Table 2). A PCR product of 1,080 bp was inserted into the pGEM-T EASY vector, yielding pFBA1-1080. A *Bgl*II/*Xho*I fragment containing the entire coding sequence of *FBA1* was isolated from pFBA1-1080 and ligated into the same sites of YPB1-ADHPt, which contain a *URA3* locus as a selectable marker of autonomously replicating sequences in *C. albicans*. The resulting plasmid, YPB1-ADHPt containing the *FBA1* ORF (pAFBA1), was transformed into CAI4 and the resulting Ura^+ transformants were selected on minimally SD agar plates.

2.6. Quinoxaline derivatives and high pressure liquid chromatography analysis (HPLC)

To determine the intracellular concentrations of α-ketoaldehydes and α-ketocarboxylic acids, the conversion into quinoxaline derivatives was performed using 1,2-diaminobenzene as previously reported, with minor modifications (Cordeiro and Freire, 1996). After the harvested cells were disrupted by 2.5 volumes of 0.5 M $HClO_4$, 1,2-diaminobenzene was added to a final concentration of 10 mM, and this mixture was incubated at 37 °C for 45 min. All quinoxaline derivatives were purified by a SPE cartridge containing C18 resin based on hydrophobicity (Waters). Quinoxaline derivatives filtered with a 0.22 μm-cellulose acetate membrane were separated by an Agilent 1200 series HPLC system with a Zorbax Eclipse XDB-C18 analytic column (4.6 × 150 mm, Agilent Technologies,

USA). The mobile phase was 67% 25 mM ammonium formate buffer, pH 3.4, 3% ACN and 30% methanol for 45 min. A total of 15 µl of each sample of was injected. The flow rate was 0.7 ml/min, and quinoxaline derivatives were detected at a wavelength of 336 nm.

2.7. Measurement of glutathione concentration

To determine the concentration of intracellular glutathione, cell extracts were reacted with monobromobimane (mBBr) to form derivates and then analyzed using a modification of method described by Newton and Fahey (1995). Cells grew in YPD medium for 16 hours and were harvested by centrifugation at 9,000 rpm for 5 min at 4 °C. Prepared cells were extracted with 50% aqueous acetonitrile (Sigma-Aldrich) containing 40 mM HEPES (pH 8.0) and 2 mM mBBr (Sigma-Aldrich). After incubation at 60 °C for 15 min, the samples were acidified with 5 µl of 5 N methanesulfonic acid (Sigma-Aldrich). Cell debris was removed from the crude extract by centrifugation at 12,000 rpm for 15 min, and the resulting supernatant was analyzed using HPLC. Control samples were treated with 10mM *N*-ethylmaleimide (NEM, Sigma-Aldrich) and incubated at 50 °C for 10 min before derivatization to prevent labeling of thiol group from with mBBr. The concentration of total GSH was determined using 2mM dithiothretol (DTT), which reduces GSSG to GSH. Samples were passed through a ZORBAX SB-C18 column (4.6 × 250 mm, Agilent Technologies, USA). HPLC was performed using a Water system equipped with a Hewlett-Packard 1050 series fluorescence detector. The mBBr-derived thiol compounds were detected using excitation and emission at 370 and 480 nm, respectively. The mobile phase consisted of buffer A (methanol, HPLC grade from Sigma-Aldrich) and buffer B (0.1% trifluoroacetic acid from Sigma-Aldrich). The proportion of buffer A in the continuous gradients was as follows; 15% at 0-2 min, 25% at 30 min, 100% at 34 min, 15% at 37 min, and 15% at 40 min. If necessary, samples were co-injected with GSH (Duchefa) standards.

2.8. RNA extraction and northern blot analysis

Total RNA from cells grown in YPD or SP broth or agar plate were prepared by the hot phenol extraction method according to the method described by Köhrer (1991). A total of 10 μg RNA from each sample was separated on a 1.0% (w/v) agarose gel containing 0.22 M formaldehyde (Sigma-Aldrich) and transferred to a Hybond-N^{+}+ nylon membrane (GE Healthcare) by electrophoresis. The specific probes were prepared by PCR and were labeled with [α-32P]-dATP for 16 h at 30 °C (Feinberg and Vogelstein, 1983). The primer sequences of each probes for hybridization were summarized in Table. Hybridization was performed using various probed dissolved in Rapid-Hyb buffer (GE Healthcare) according to the manufacturer's instructions. The blots were incubated in Rapid-Hyb buffer without the probe for 1 h and then probe was added for 2h at 65 °C. The blot was washed twice with SSC buffer (0.1% SS, 0.3M NaCl, 30mM trisodium citrate) for 10 min. The signal was developed by the bio-imaging system BAS-2500 (FujiFilm).

2.9. Measurement of intracellular reactive oxygen species (ROS) concentration

All strains were grown in YPD or Spider medium at 28 °C. 2 x 10^{7} cells were collected by diluting into 25 mM Tris-HCl, pH 8.5. 20 μM dichloroflurescin diacetate, DCFH-DA, was added to measure intracellular ROS concentration according to the method described by Shen *et al*. (2006). Samples were suspended in a 96-well plate. To examine, a Cary Eclipse Fluorescence Spectrophotometer (Varian) was used. ROS was detected using excitation and emission at 492 and 524 nm, respectively.

2.10. Cell cycle analysis

Cell cycle analysis was performed by quantifying DNA content via flow cytometry using cells stained with PI (propidium iodide, 50 μg/mL) as described previously,

with minor modifications (Haase and Reed, 2002). All strains were grown in YPD or SPider medium at 28 °C. After 2×10^6 cells were collected, the sorted populations of *C. albicans* strains were washed with PBS and resuspended dropwise in 1 ml of 70% ice-cold ethanol in phosphatase based saline (PBS). After an overnight incubation at 4 °C, *Candida* cells were collected by centrifugation at 5,000 × *g* for 10 min, suspended in a solution of bovine pancreas RNase (DNase free, 200 μg/ml), and incubated at 28 °C for 30 min. Cells were centrifuged and resuspended in PI solution (50 μg/ml in 0.05 % Triton X-100 in PBS) and stained for 30 min in the dark. The DNA content was measured with a FACS Calibur flow cytometer (Becton Dickinson). An output of 0.15 W of 488 nm light was used for excitation and fluorescence was determined at wavelength above 620 nm. All flow cytometric acquisitions and analyses were performed using CELL Quest Pro data acquisition and analysis software, and the percentage of the cell population in the G1 and G2 phases was determined using Modfit LT 3.0.

III. RESULTS

1. Cloning and characterization of *FBA*1 in *C. albicans*

In order to investigate the roles of *FBA1* in *C. albicans*, full length of *FBA1* gene was amplified by PCR using primer *FBA1* as described in Table 3. The ORF of *FBA1* encoded a polypeptide consisting of 360 amino acids with a calculated molecular mass of 39,215 Da (Fig. 1). *C. albicans FBA1* showed 73% of identity over entire sequence with *S. cerevisiae FBA1* by BLAST searches of the GenBank database. *C. albicans FBA1* contained no CUG codons, which encodes serine in *C. albicans* but encodes leucine in *S. cerevisiae* and elsewhere (Santos and Tuite, 1995) (Fig 2a and 2b). The nucleotide sequences of FBA1 had no consensus sequence for splicing (Fig. 2a and 2b).

2. Disruption and reintegration of *C. albicans FBA1*

In vitro constructs, plasmid pQF181-SHF forward-oriented and pQF182-SHR containing reverse-oriented cloned from pQF181 and pQF182 were prepared by inserting the *hph-URA3-hph* sequence into a portion of the encoding region of *FBA1* (Fig. 3a). Digestion of pQF181-SHF and pQF182-SHR with *Sac*I/*Hind*III released a 4.5-kb fragment, which was used to transform CAI4 Ura^- strain, respectively (Fig. 3b). Transformation was performed according to modified LiAc method of Walther and Wendlend (2003). The resulting Ura+ transformants were selected on FOA medium and confirmed by PCR and northern analysis (Fig. 4). A homozygous disruption of *FBA1* could not generate. It was not viable. Transformants were immediately died right after forming a colony.

3. *C. albicans FBA1* overexpression

In order to overexpress FBA1 in *C. albicans*, the plasmid pAFBA1 was constructed by inserting the FBA1 coding region into the plasmid YPB1-ADHPt (Csank, 1998) as described Materials and Methods. The resulting construct was transformed to *C. albicans* CAI4, isogenic strain with the parent plasmid YPB1-

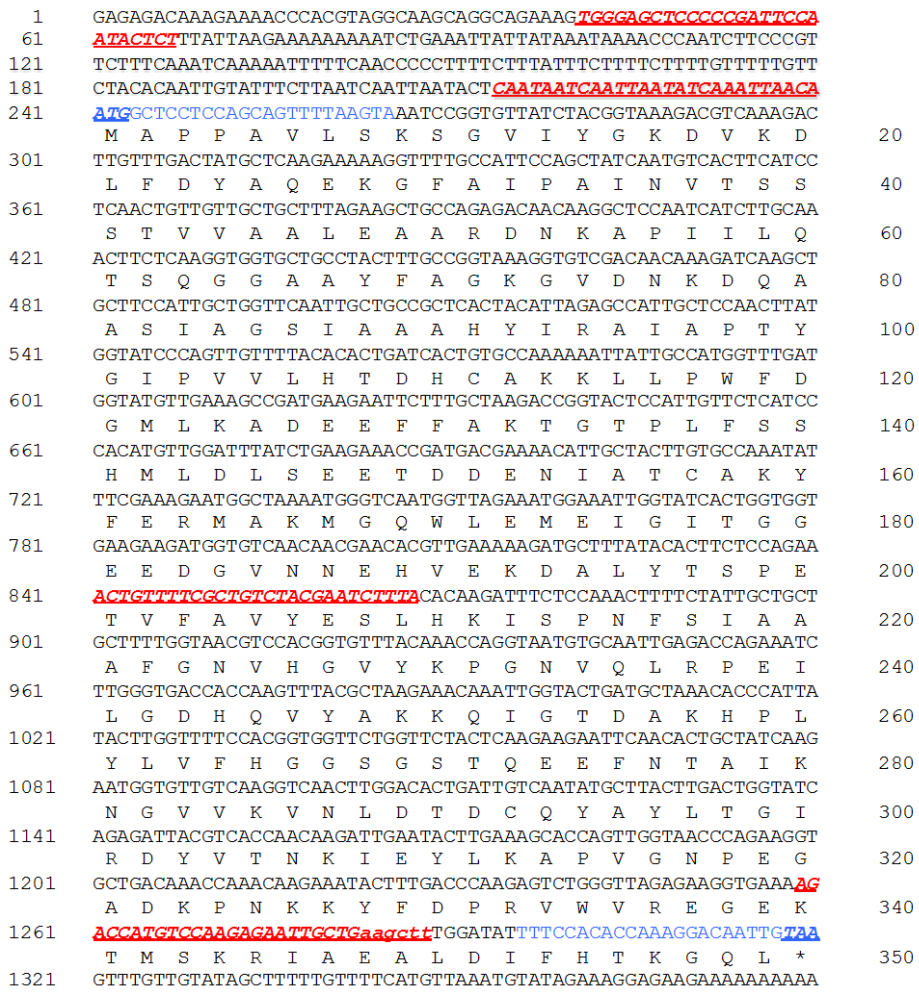


Fig. 1. Nucleotide and deduced amino acid sequence of an ORF CaO19.4618 (*Candida albicans* genome database : http://www.candidagenome.org/), which has been identified as *fructose 1,6-bisphosphate aldolase 1 (FBA1)* in *C. albicans*. The nucleotides are numbered on the left respectively. The termination codon is indicated with an asterisk. The dashed lines indicate oligonucleotide primers used for cloning, disruption *FBA1* by PCR.

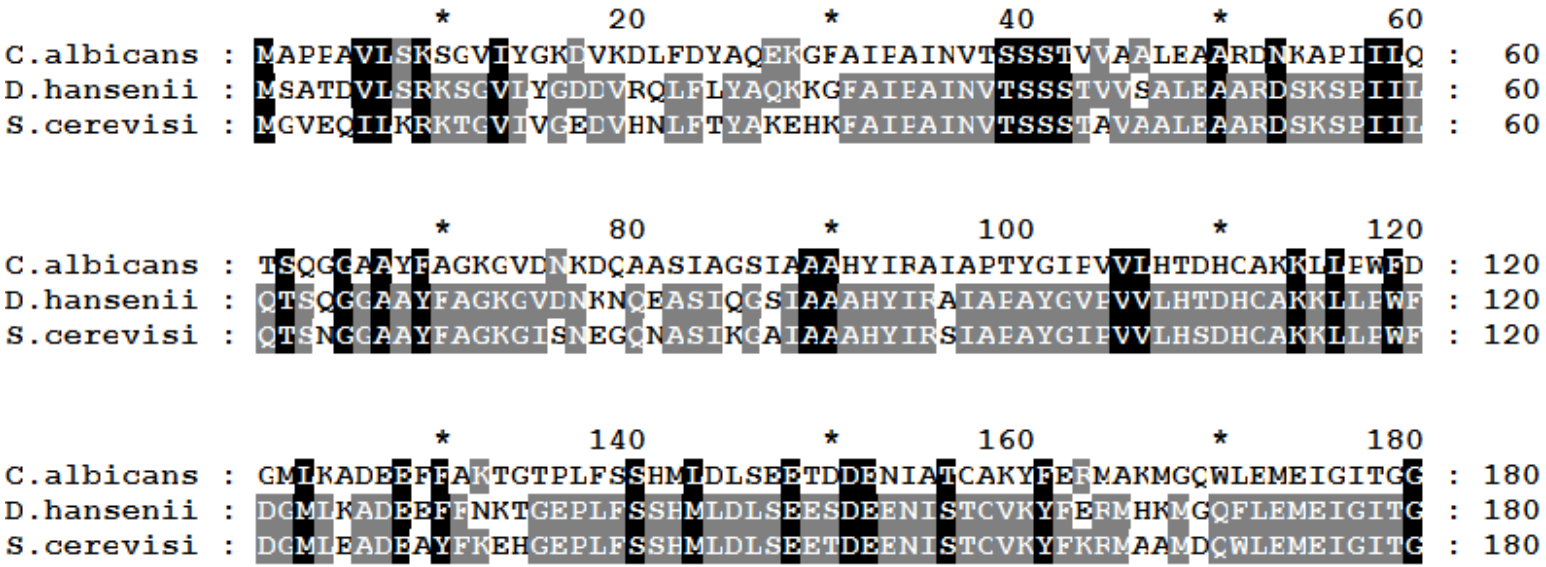


Fig. 2a. The sequence alignment of the deduced amino acids of fructose 1,6-bisphosphate.

The amino acid sequence deduced from the *Candida albicans FBA1* gene was aligned with other species using vector NTI 9.0 explorer clustal X program. Identical amino acid residues, conservative, block of similar and weakly similar amino acid residues are indicated with shaded letters. The numbers on the top refer to the amino acid positions in an each sequence. *C. albicans, Candida albicans*; D. hansenii, *Debrayomyces hansenii*; S. cerevisi, *Saccharomyces cerevisiae*.

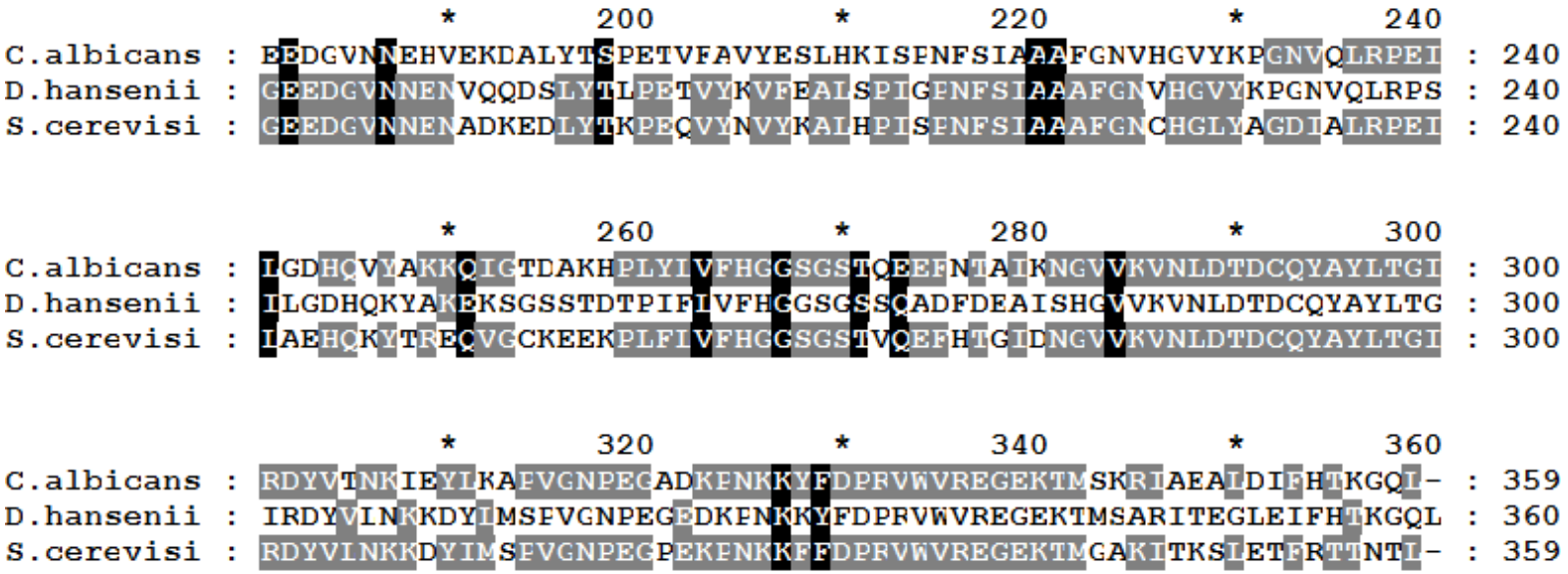


Fig. 2b. The sequence alignment of the deduced amino acids of fructose 1,6-bisphosphate.

The amino acid sequence deduced from the *Candida albicans FBA1* gene was aligned with other species using vector NTI 9.0 explorer clustal X program. Identical amino acid residues, conservative, block of similar and weakly similar amino acid residues are indicated with shaded letters. The numbers on the top refer to the amino acid positions in an each sequence. *C. albicans*, *Candida albicans*; D. hansenii, *Debrayomyces hansenii*; S. cerevisi, *Saccharomyces cerevisiae*.

Table 2. Plasmids used in this study.

Plasmid	Descriptions	Sources or references
pGEM-T easy	PCR cloning vector; Ap^r	Promega
YPB1-ADHPt	*ADH1* promoter and terminator in *URA3* - marked 2 μm vector (Expression vector of *Candida albicans*)	Csank *et al*., 1998
p5912	*hisG-URA3-hisG* cassette in pUC18	Fonzi and Irwin, 1993
pQF18	*hph-URA3-hph* cassette in pBluescript KS+	Feng *et al*., 1999
pQF181	pGEM-T easy vector containing *hph*-URA3-*hph* cassette which can be excised with *Sac*I/*Hind*III (Forward)	Hwang *et al*., 2003
pQF182	pGEM-T easy vector containing *hph*-URA3-*hph* cassette which can be excised with *Sac*I/*Hind*III (Reverse)	Hwang *et al*., 2003
pQF181-SK	pGEM-T easy vector containing *hph*-URA3-*hph* cassette with upstream flank region of *FBA1* ORF	This work
pQF181-SH	pGEM-T easy vector containing *hph*-URA3-*hph* cassette with downstream flank region of *FBA1* ORF	This work
pQF181-SHF	pGEM-T easy vector containing *hph*-URA3-*hph* cassette with upstream and downstream flank region of FBA1 ORF	This work

pQF182-SK	pGEM-T easy vector containing *hph*-URA3-*hph* cassette with upstream flank region of *FBA1* ORF	This work
pQF182-SH	pGEM-T easy vector containing *hph*-URA3-*hph* cassette with downstream flank region of *FBA1* ORF	This work
pQF182-SHF	pGEM-T easy vector containing *hph*-URA3-*hph* cassette with upstream and downstream flank region of FBA1 ORF	This work
pFBA1-1080	pGEM-T easy vector containing *FBA1* ORF	This work
pAFBA1	*ADH1* promoter:: *FBA1* in YPB1-ADHPt	This work

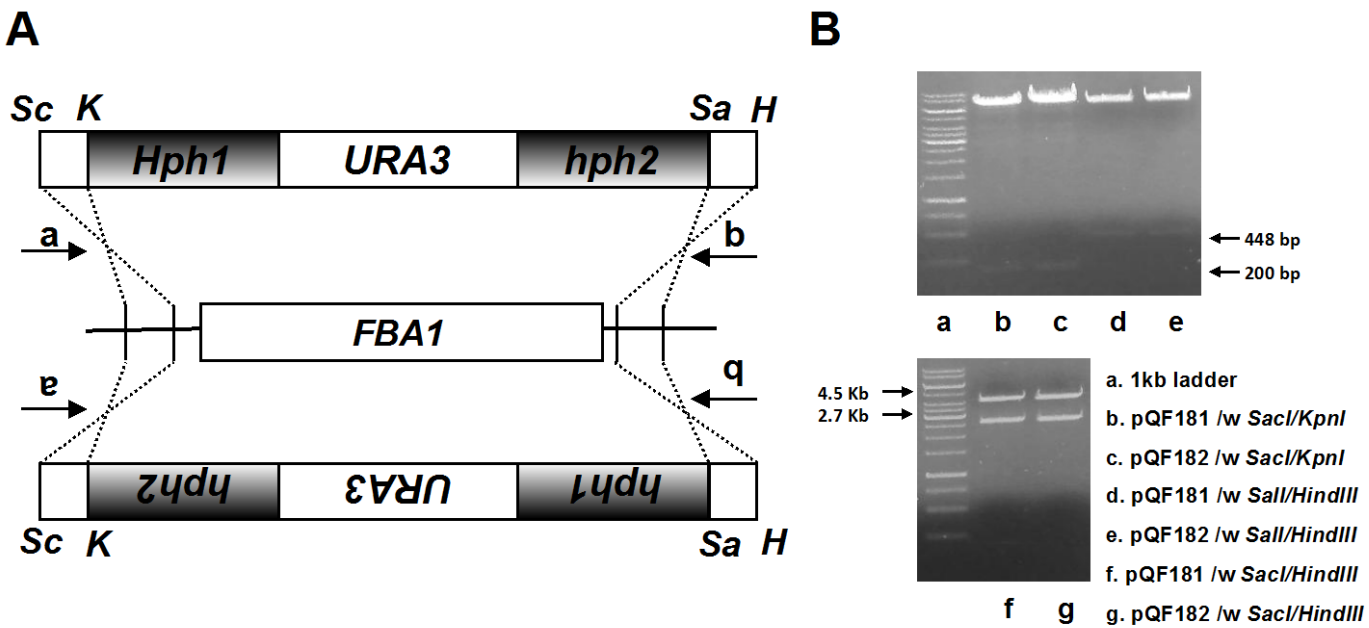


Fig. 3. Strategies for sequential disruption of *fructose 1,6-bisphosphate aldolase 1* (*FBA1*). Creation of "URA-baslter method" was used to insertion of the hph-URA3-hph cassette according to Fonzi and Irwin (1992) (A) and reintegration of wild-type *FBA1* into the *fba1/fba1* disruptant (B) in *C. albicans.*

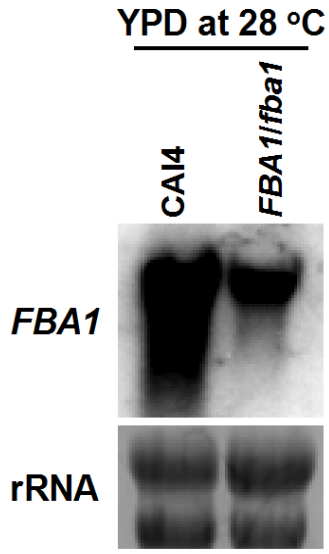


Fig. 4. RNA profiles of fructose-1,6-bisphosphate (*FBA1*) gene expression by Northern blot analysis. 20μg of total RNA of *Candida albicans* cells grown on YPD media at 28 °C for 16 hours were extracted by hot phenol methods as previously described. Total RNA of wild type and heterozygous disruptant (*FBA1/fba1*) cells were hybridized with *FBA1* DNA probe-s labeled with α-^{32}P radioactive isotope using random primin according to appropriate procedures. Strains used in this experiment were as follows: lane 1, CAI4, wild type isolate; lane 2, *Δura3::FBA1/fba1*, heterozygous disruptant, respectively.

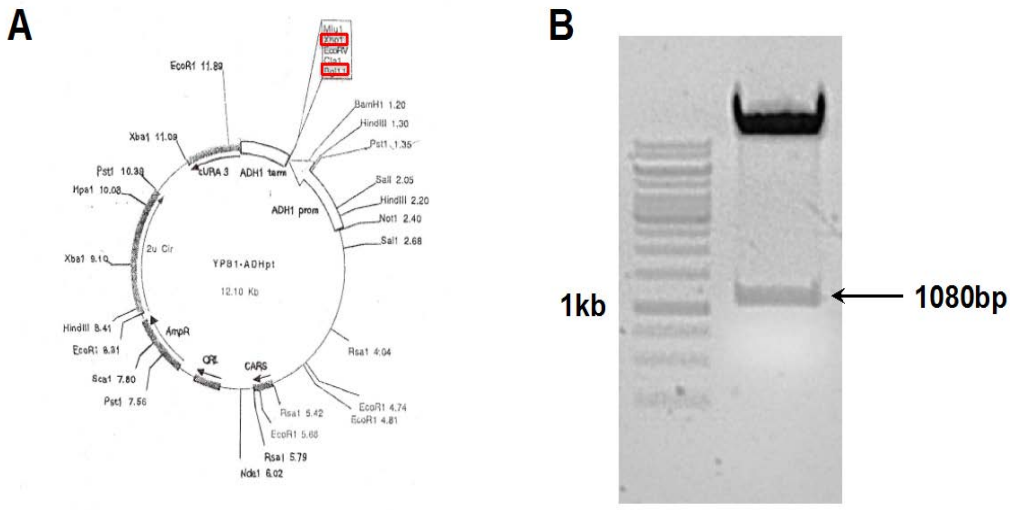


Fig. 5. *FBA1* overexpression in *C. albicans*. (A) The vector construct for FBA1 overexpression in *C. albicans*. Red indicates the restriction enzyme sites which used for insert ORF of *FBA1* (B) The FBA1 genomic DNA was inserted with *Bgl*II and *Xho*I enzymes sites into the original YPB1-ADHpt vector.

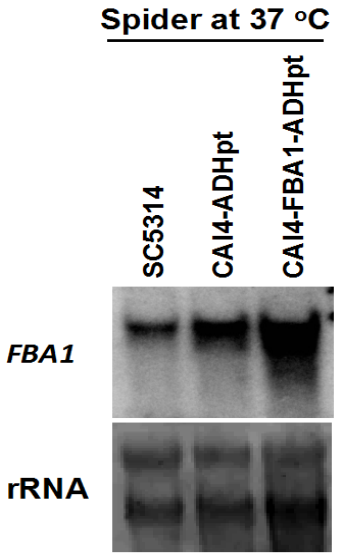


Fig. 6. RNA profiles of fructose-1,6-bisphosphate (*FBA1*) gene expression by Northern blot analysis. 20 µg of total RNA of *Candida albicans* cells grown on spider agar medium at 28 °C for 3 days were extracted by hot phenol methods as previously described. Total RNA of wild type and over-expressed cells were hybridized with *FBA1* DNA probes labeled with α -^{32}P radioactive isotope using random priming according to appropriate procedures. Strains used in this experiment were as follows: lane 1, SC5314, wild type isolate; lane 2, CAI4-ADHPt; lane 3, CAI4-ADHPt-FBA1, respectively.

ADHPt as control and pAFBA1. Transformants were selected by plating on uracil-deficient medium for 3 days. Selected transformants were confirmed by PCR and northern blot analysis for observing expression (Fig. 5 and 6).

4. Morphological characteristics of *Fba1/fba1* mutant and *FBA1* overexpressed cell.

Colony and cell morphology were observed to determine whether *FBA1* affects the morphology of cells. To observe morphology of cells, all strains were grown on solid media that induce hyphal growth, Spider medium (Liu *et al*., 1994), and normal growth, YPD medium. SC5314 (the parental wild-type), KU101 (*fba1/FBA1*), and KU103 (CAI4-ADHpt) showed budding yeast forms of colony morphology at both 28 °C and 37 °C on YPD medium agar plates (Fig. 7 and 8). In contrast, KU104 (CAI4-ADHpt-FBA1) showed emerging filament at 28 °C and formed little hypha on the border of ovoid at 37 °C (Fig. 7 and 8). When cells were grown on solid Spider medium agar plates, SC5314 and KU103 formed wrinkled colony with stubby hyphae at both 28 °C and 37 °C (Fig. 9 and 10). In addition to that, KU101 formed wrinkled colony with stubby hyphae little smaller than SC5314 at 28 °C (Fig. 9) and wrinkled colony with flare out hyphae at 37 °C (Fig. 10). On the contrary, interestingly, KU104 showed defect in hyphal formation much smaller than budding yeast form of SC5314 at both 28 °C and 37 °C (Fig. 9 and 10). In addition to that, cell morphological characteristics were consistent with the colony morphology as described (Fig. 11 and 12). These results suggest that *FBA1* gene seems to be involved in activation/inhibition of the genes for hyphal formation.

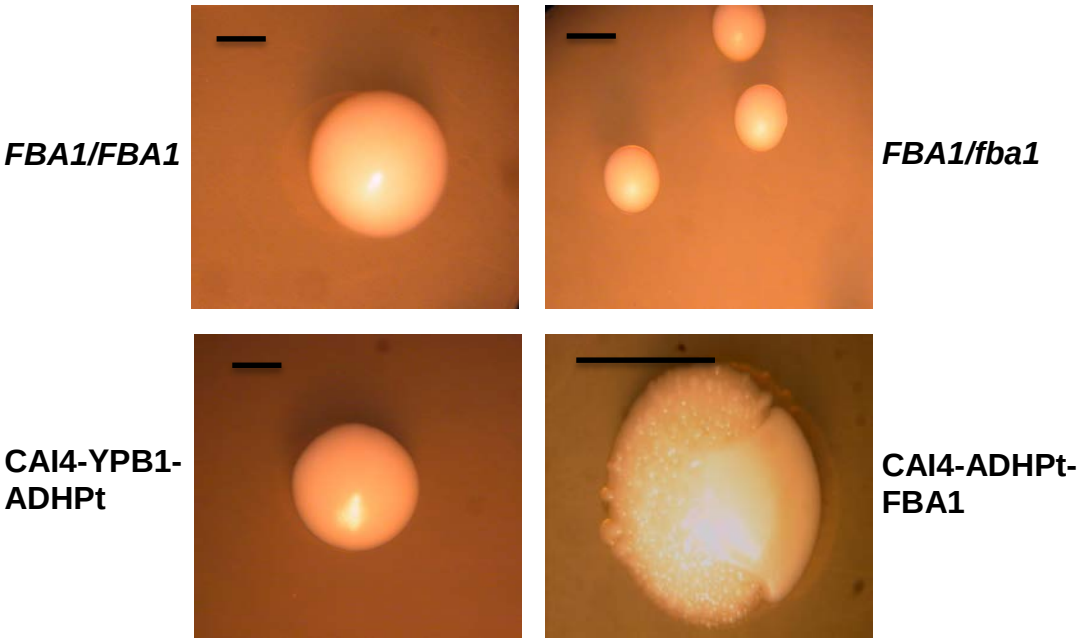


Fig. 7. Colony-morphology change of the *FBA1/fba1* and over-expression of *FBA1* in *C. albicans* on YPD at 28 °C.

SC5314 (wild type; FBA1/FBA1), KU101 (FBA1/fba1), KU103 (CAI4-YPB1-ADHPt), and KU104 (CAI4-ADHPt-FBA1) strains were plated on YPD medium for 3 days at 28 °C. Scale bar represents 1mm.

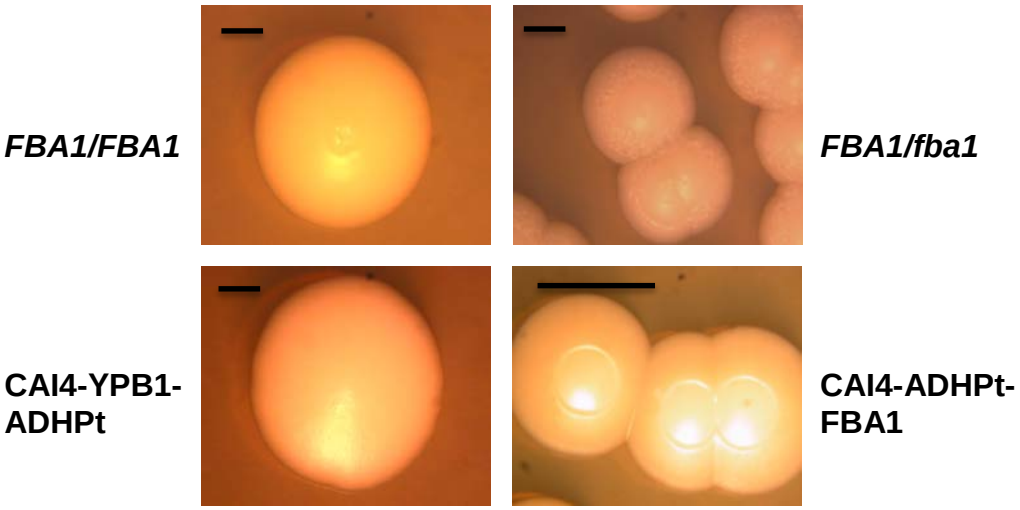


Fig. 8. Colony-morphology change of the *FBA1/fba1* and over-expression of *FBA1* in *C. albicans* on YPD at 37 °C.

SC5314 (wild type; FBA1/FBA1), KU101 (FBA1/fba1), KU103 (CAI4-YPB1-ADHPt), and KU104 (CAI4-ADHPt-FBA1) strains were plated on YPD medium for 3 days at 37 °C. Scale bar represents 1 mm.

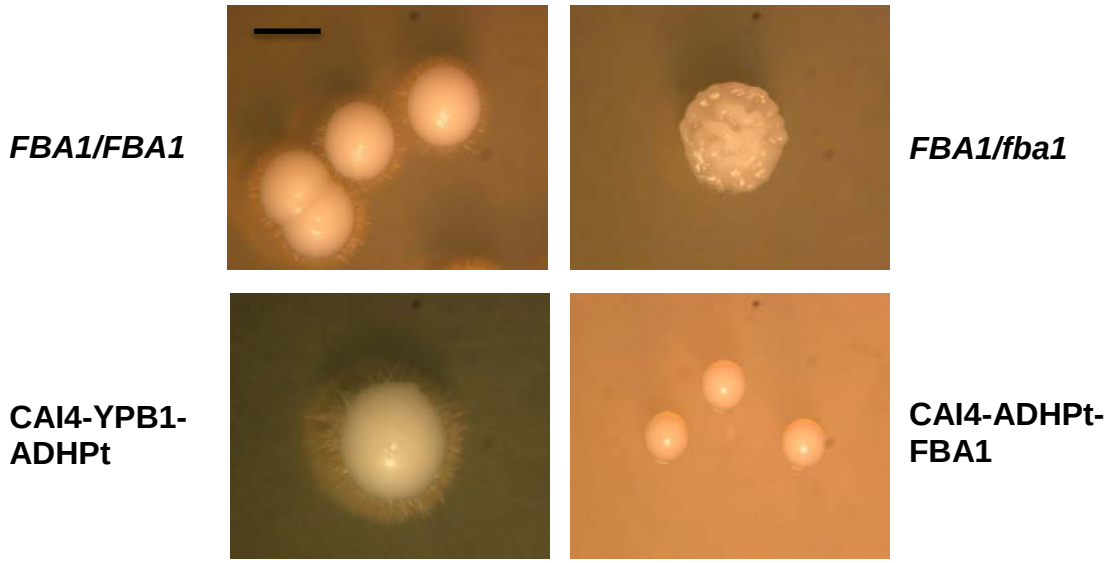


Fig. 9. Colony-morphology change of the *FBA1/fba1* and over-expression of *FBA1* in *C. albicans* on spider at 28 °C.

SC5314 (wild type; FBA1/FBA1), KU101 (FBA1/fba1), KU103 (CAI4-YPB1-ADHPt), and KU104 (CAI4-ADHPt-FBA1) strains were plated on Spider medium for 3 days at 28 °C. Scale bar represents 1mm.

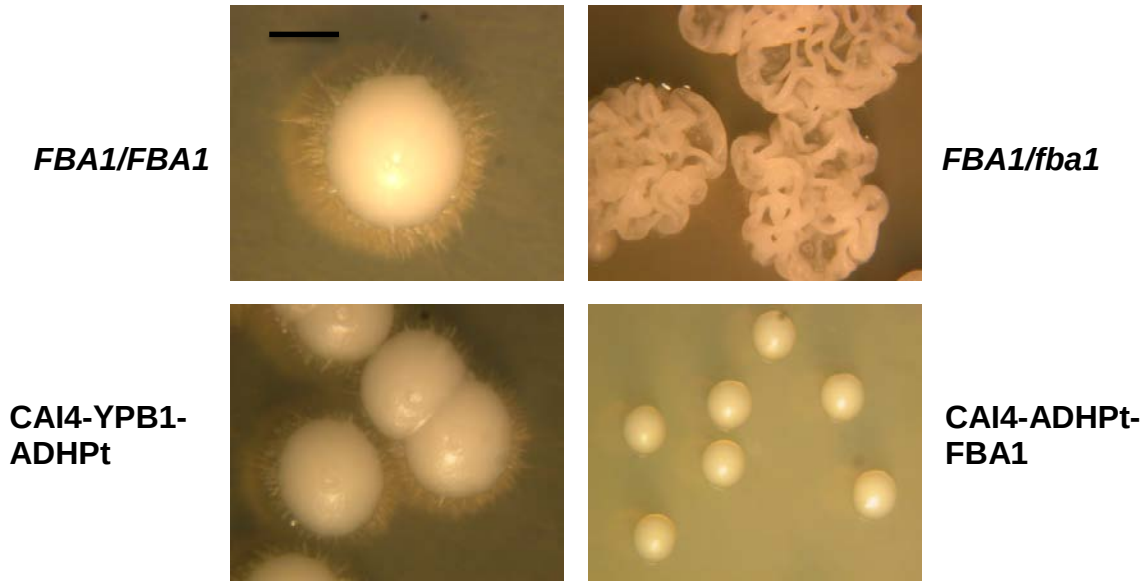


Fig. 10. Colony-morphology change of the *FBA1/fba1* and over-expression of *FBA1* in *C. albicans* on spider at 37 °C.

SC5314 (wild type; FBA1/FBA1), KU101 (FBA1/fba1), KU103 (CAI4-YPB1-ADHPt), and KU104 (CAI4-ADHPt-FBA1) strains were plated on Spider medium for 3 days at 37 °C. Scale bar represents 1mm.

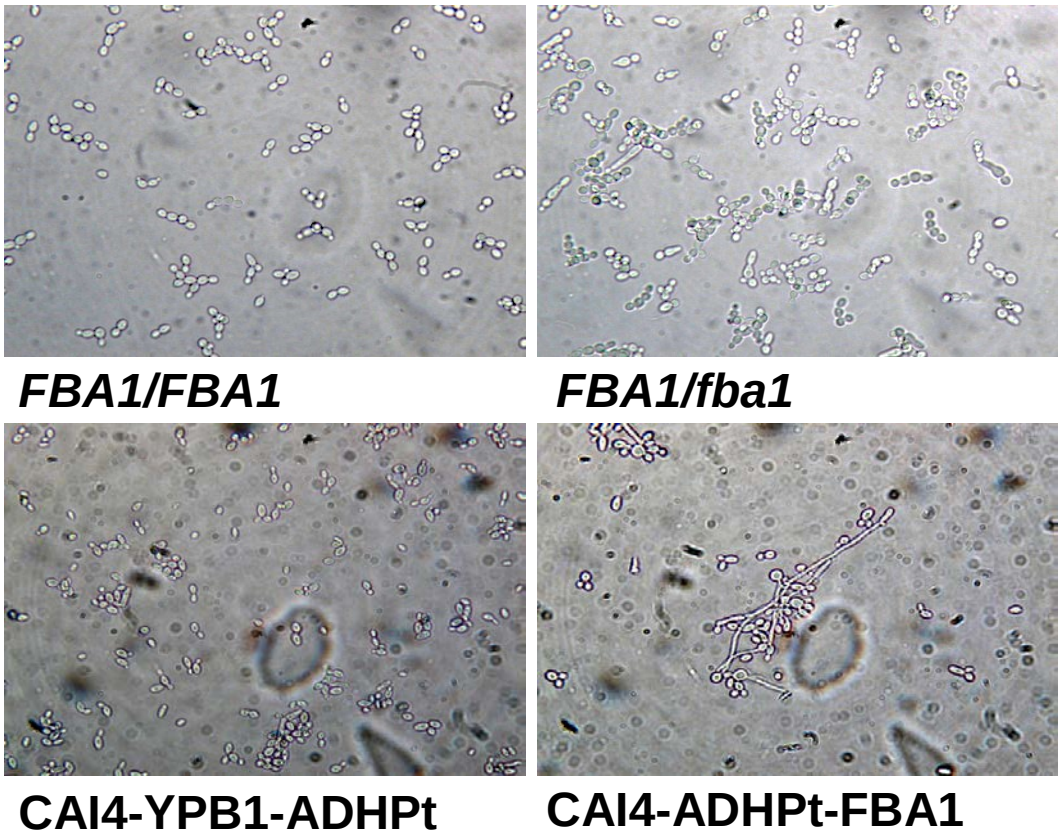


Fig. 11. Morphological characteristics of fructose-1,6-bisphosphate aldolase mutant of *C. albicans* in YPD medium.

SC5314 (wild type: FBA1/FBA1), KU 101 (FBA1/fba1), KU 103 (CAI4-YPB1-ADHPt), and KU 104 (CAI4-ADHPt-FBA1) strains were incubated with initial O.D=0.1 on YPD medium for 4 hours at 28 °C. Photos were taken under 400X magnification.

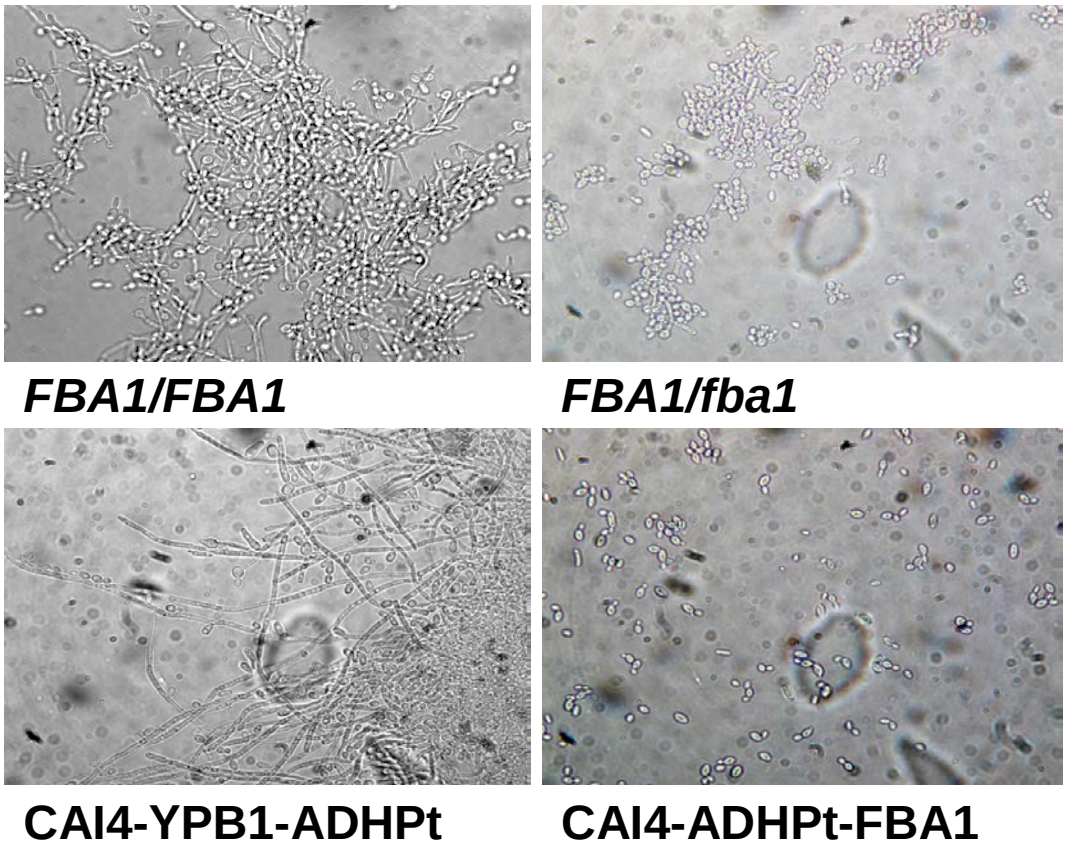


Fig. 12. Morphological characteristics of fructose-1,6-bisphosphate aldolase mutant of *C. albicans* in Spider medium.

SC5314 (wild type: FBA1/FBA1), KU 101 (FBA1/fba1), KU 103 (CAI4-YPB1-ADHPt), and KU 104 (CAI4-ADHPt-FBA1) strains were incubated with initial O.D=0.1 on Spider medium for 4 hours at 37 °C. Photos were taken under 400X magnification.

5. Effects of disruption and overexpression of *FBA1* on the expression of hypha-specific and virulence genes in *C. albicans*.

To understand the effects of FBA1 mutation more clearly, the expressions of genes related to hyphal formation and virulence such as ECE1, EFG1, HWP1, ALS1, and TUP1 were compared by northern analysis (Table 3). As expected, the expression patterns of filament specific gene were consistent with phenotype of mutant cells. The filament specific mRNAs-ECE1, HWP1, and EFG1 were not expressed in SC5314 and KU103 in YPD medium at 28 °C, while level of these genes were elevated in FBA1-overexpressing mutant, KU104 (Fig. 13). When cells were grown in spider medium, expression levels of filamentous specific genes were increased but not in FBA1-overexpressing mutant were not detected (Fig. 13).

6. Relation between *FBA1* and intracellular glutathione (GSH)

To investigate the relation between GSH and *FBA1*, the concentration of intracellular GSH was measured in KU101 (*fba1/FBA1*) and KU104 (CAI4-ADHpt-FBA1) cells (Fig. 16). In KU101 cells, the concentration of intracellular GSH was 13.5 % greater than SC5314 cells in YPD medium (Fig.16). In KU104 cells, the concentration of intracellular GSH were 69.8 % lower than SC5314 and 83.6 % lower than KU103 (CAI4-ADHpt) in YPD medium (Fig. 16). On the contrary, in KU101 and KU104 cells, the concentration of intracellular GSH were 527.1 % and 411.6 % higher than SC5314 and 500 % and 364 % higher than KU103 in Spider medium (Fig. 16). These data indicate that the concentration of intracellular GSH was affected by *FBA1* expression in *C. albicans*.

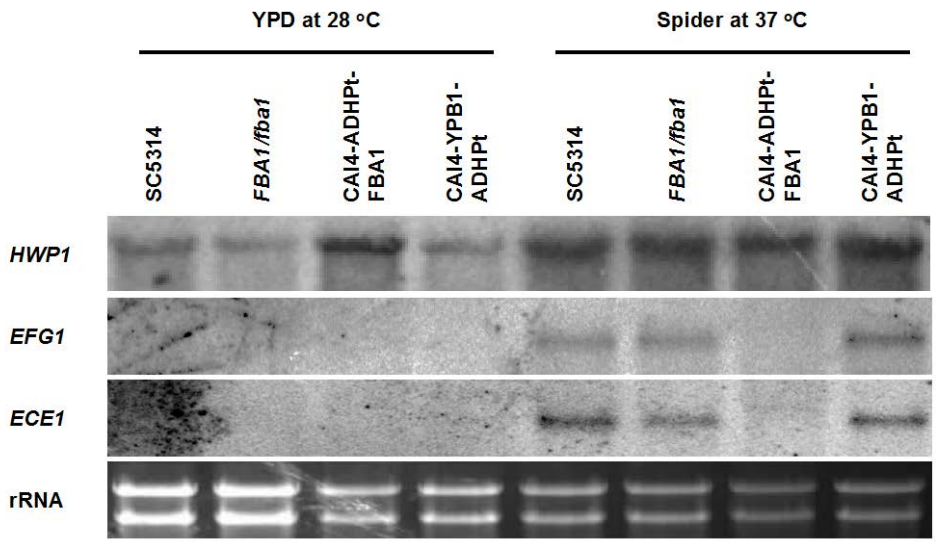


Fig. 13. Expression of regulators in filamentation by northern blot analysis.

20 μg of total RNA of *Candida albicans* cells grown on YPD medium at 28°C or spider medium at 37°C for 3 days were prepared from each strain, and northern analysis was carried out with probes to the indicated genes. Strains used in this experiment were as follows: lane 1,5, SC53134 (*FBA1/FBA1*), wild type isolate; lane 2,6, KU101 (*FBA1/fba1*), heterozygous disruptant; lane 3,7, KU104 (CAI4-ADHPt-FBA1); lane 4,8, KU103 (CAI4-YPB1-ADHPt), respectively.

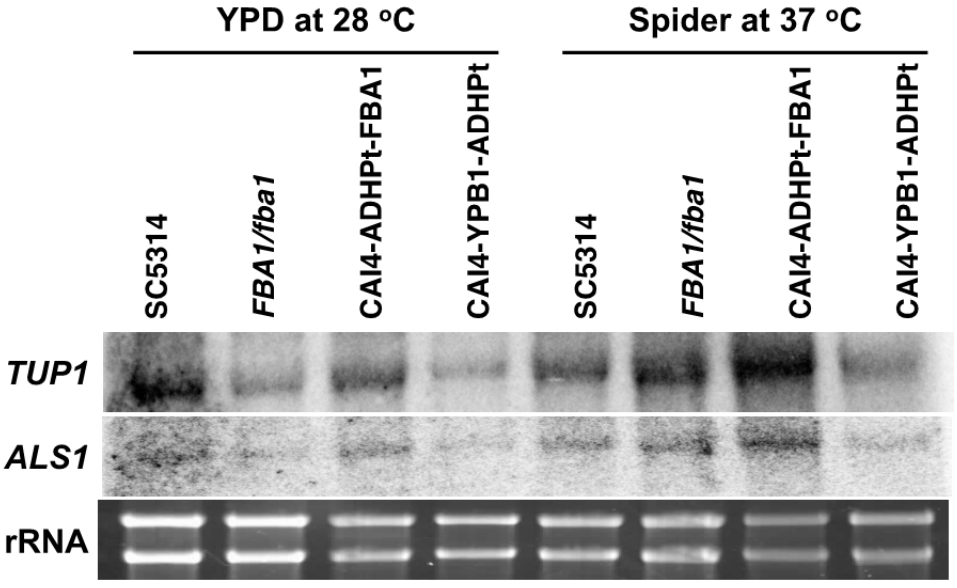


Fig. 14. Expression of regulators in filamentation by northern blot analysis.

20μg of total RNA of *Candida albicans* cells grown on YPD medium at 28 °C or spider medium at 37 °C for 3 days were prepared from each strain, and northern analysis was carried out with probes to the indicated genes. Strains used in this experiment were as follows: lane 1,5, SC53134 (*FBA1/FBA1*), wild type isolate; lane 2,6, KU101 (*FBA1/fba1*), heterozygous disruptant; lane 3,7, KU104 (CAI4-ADHPt-FBA1); lane 4,8, KU103 (CAI4-YPB1-ADHPt), respectively.

Table 3. List of primer sequences used in PCR and northern blot analysis for the preparation of hybridization probes.

	Primer sequences (5' to 3')	
For PCR		
FBA1	F: ATGGCTCCTCCAGCAGTTTTAAGTAAATCC R: GATATTTTCCACACCAAAGGACAATTGTAA	
*FBA1*_SK	F: TGGGAGCTCCCCCGATTCCAATACTCT R: CAATAATCAATTAATATCAAATTAACA	
*FBA1*_SH	F: ACTGTTTTCGCTGTCTACGAATCTTTA R: AGACCATGTCCAAGAGAATTGCTG	
For probe		**Position**
EFG1	F: ATGTCAACGTATTCTATACCCTAT R: GAGGAGCCGAAGCAGAAGTGGCAG	1 to 1000
ECE1	F: ATGAAATTCTCCAAAATTGCCTGT R: TTAAGCTTTTCCGAAATATTCTTC	1 to 816
HWP1	F: ATGAGATTATCAACTGCTCAACTT R: TAACACCAGTAGTAACTTCACTTT	1 to 1000
TUP1	F: ATGTCCATGTATCCCCAACGCACC R: AACACTGGGAGGTTCTCAAGTCCC	1 to 1000
ALS1	F: ATGCTTCAACAATTTACATTGTTA R: CTGTTCTAGTTGTAGCAACAATGA	1 to 1000

F, forward primer; R, reverse primer.

7. Relation between *FBA1* and intracellular methylglyoxal (MG)

To investigate the relation between MG and *FBA1*, the concentration of intracellular MG was measured in KU101 *(fba1/FBA1*) and KU104 (CAI4-ADHpt-FBA1) cells (Fig.15) in YPD and Spider medium. In KU101 cells, the concentration of intracellular MG increased by 103.2 and 144.06 compared to
SC5314 in YPD and spider medium, respectively (Fig. 15). In KU104 cells, the concentration of intracellular MG increased by 192.8 and 179.3 compared to SC5314 and KU103 (CAI4-ADHpt) in YPD medium (Fig.15). On the contrary, in spider medium, the concentration of intracellular MG in KU104 decreased by 168.06 and 185.43 compared to SC5314 and KU103 (Fig. 15). These data suggested that overexpression of *FBA1* led to accumulation of MG especially in dextrose containing medium which cells use as a carbon source.

8. Relation between *FBA1* and intracellular reactive oxygen species (ROS)

To determine the relation between *FBA1* and ROS, a fluorescence spectrophotometer was used with DCFH-DA dye. The intracellular level of ROS was measured in KU101 (*fba1/FBA1*) and KU104 (CAI4-ADHpt-FBA1) cells. In KU101, the level of intracellular ROS was increased by 48.17 in YPD medium and similar in spider medium compared to SC5314 (Fig. 17). In KU104, the level of intracellular ROS showed significant increased by 67.1 and 66.66 in YPD medium compared to SC5314 and KU103 (CAI4-ADHpt), respectively (Fig. 17). On the contrary, in KU104, the level of intracellular ROS decreased by 38.3 and 39.5 in spider medium compared to SC5314 and KU103, respectively (Fig. 17). These data suggested that overexpression of *FBA1* led to intracellular ROS to increase which consists with the result of intracellular MG level in YPD medium.

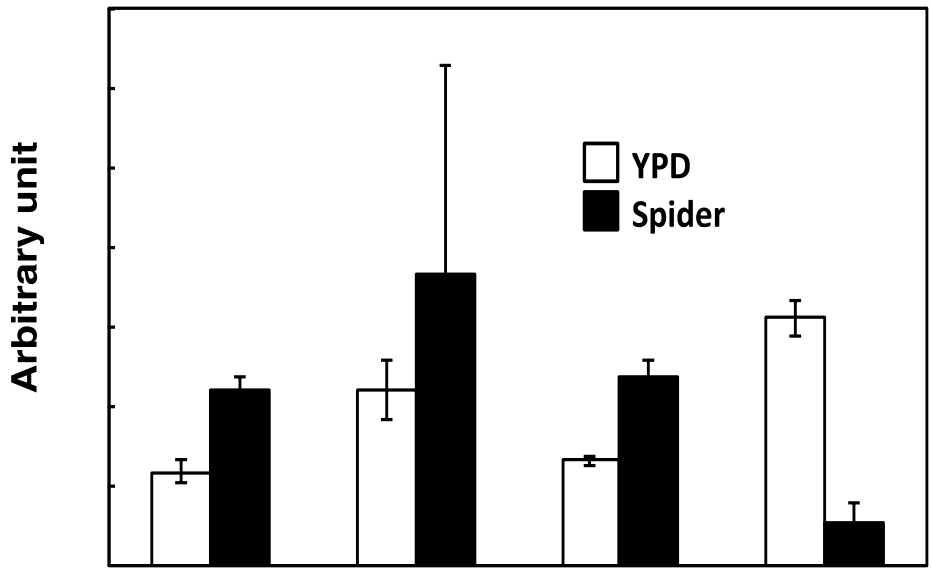


Fig. 15. The intracellular methylglyoxal (MG) concentration in *FBA1*-overexpressing cells in *C. albicans*.

The concentration of intracellular MG was measured in exponentially growing SC5314 (*FBA1/FBA1*), KU101 (f*ba1/FBA1*), KU103 (CAI4-YBP1-ADHpt), and KU104 (CAI4-ADHpt-FBA1) cells using HPLC at 336 nm. Intracellular MG as modified to an quinoxaline derivatives form to detect. The concentration of MG was calculated in relative values compared to that of SC5314 cells. The values represent the mean ± S.E.M. of three independent experiments.

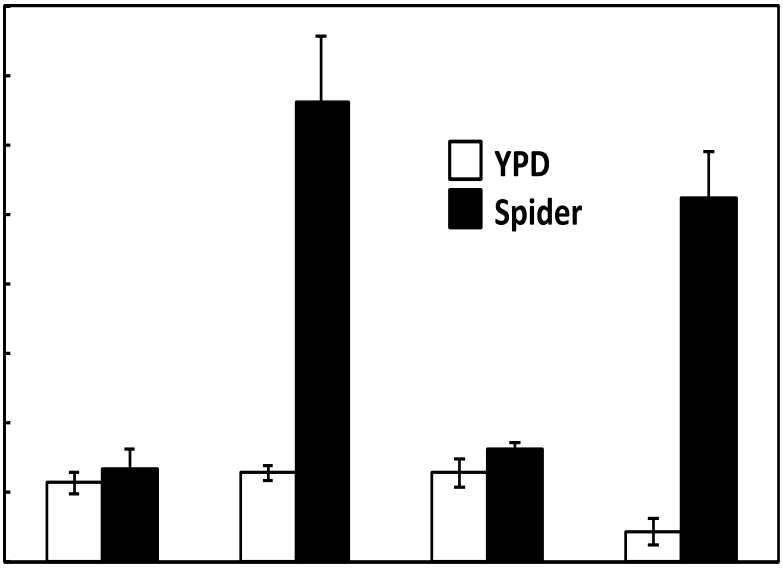


Fig. 16. The intracellular glutathione (GSH) concentration in *FBA1*-overexpressing cells in C. albicans.

The concentration of intracellular GSH was measured in exponentially growing SC5314 (*FBA1/FBA1*), KU101 (f*ba1/FBA1*), KU103 (CAI4-YPB1-ADHpt),and KU104 (CAI4-ADHpt-FBA1) cells using HPLC and fluorescent detector. Intracellular GSH as modified to a mBBr-conjugated form to detect. The concentration of GSH was calculated in relative values compared to that of SC5314 cells. The values represent the mean ± S.E.M. of three independent experiments.

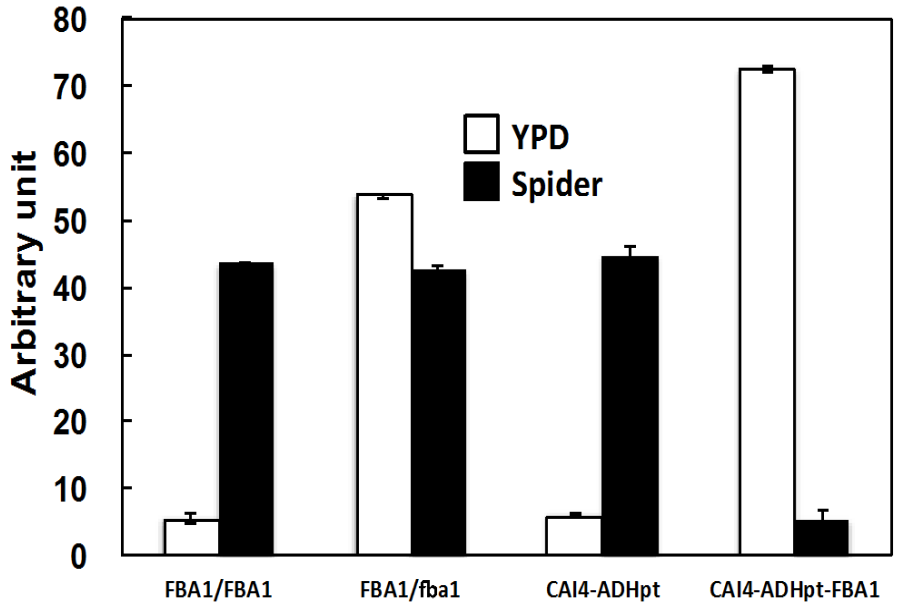


Fig. 17. The intracellular reactive oxygen species (ROS) concentration in FBA1-overexpressing cells in *C. albicans*.

The concentration of intracellular ROS was measured in exponentially growing SC5314 (*FBA1/FBA1*), KU101 (f*ba1/FBA1*), KU103 (CAI4-YPB1-ADHpt), and KU104 (CAI4-ADHpt-FBA1) cells using a fluorescence spectrophotometer. Intracellular ROS was detected with DCFH-DA. DCFH-DA cleaved when ROS produced. The values represent the mean ± S.E.M. of three independent experiments.

9. Cell cycle analysis using propidium iodide by Flow Cytometry

To observe relative DNA content in FBA1 overexpressing mutant, flow cytometry was used with propidium iodide solution. Figure # clearly showed that G1 arrest occurred in KU101(*fba1/FBA1*) and KU104 (CAI4-ADHpt-FBA1) cells compared to SC5314 (wild type) and KU103 (CAI4-ADHpt) in YPD. More specifically, KU101 showed 61.99 % of G1 cells and 19.99 % of G2 cells and KU104 appeared to show 56.31 % of G1 cells and 21.46 % of G2 cells (Fig. 18). Whereas SC5314 presented 32.47 % of G1 cells and 38.19% of G2 cells and KU103 presented 34.53 % and 39.93 % of G1 and G2 cells respectively (Fig. 18). These data clearly indicate that overexpression of FBA1 led to G1-phase arrest when the strain grows in YPD medium.

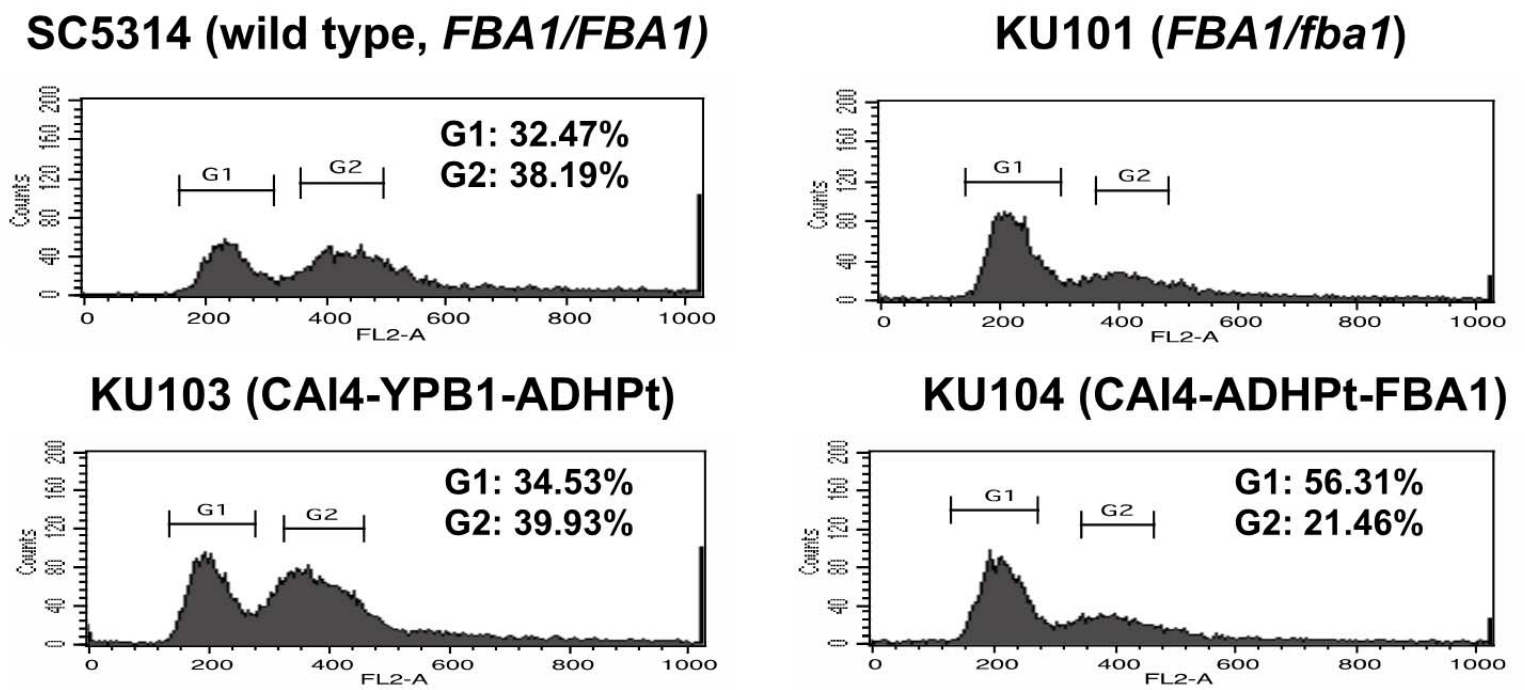
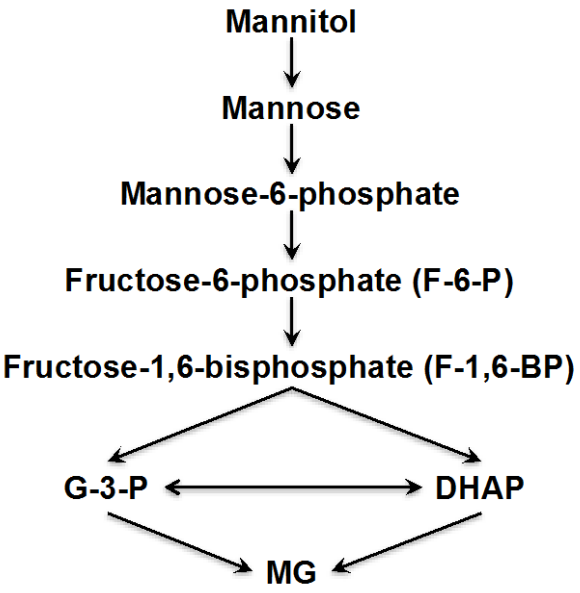


Fig. 18. Cell cycle analysis using propidium iodide by Flow Cytometry in *C. albicans*.

The DNA levels in FBA1 disruptant and overexpressing cells were analyzed through flow cytometry as described. The cells were used as reference experiments and as seeding cells, an optical density at 600 nm of 0.05 in SD, YPD or spider medium at 28 °C. The numbers indicate the percentage of G1/G2 cells.



Scheme 3. Mannitol degradation pathway.

The mannitol degradation pathway and how it might connect to the intermediate products of glycolytic pathway.

IV. DISCUSSION

In general, methylglyoxal (MG) is thought to be an important compound, which exists in most organisms such as mammals, yeast, plants, parasites, and prokaryotes (Kakapos, 1999). It is known to a toxic compound, which generates free radical and advanced glycation end product by non-enzymatic elimination (Chan *et al*., 2007). Although it has been suggested that MG is derived from glucose (Kalapos, 1999), not much studied had been done. More specifically, where MG is controlled and how it affects to cells are obscure.

In this work, fructose-1,6-bisphosphate aldolase, one of glycolytic enzymes, is selected to study since it cleaves fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate which leads to production of MG directly (Scheme 1). Therefore, the decision was made to start from fructose-1,6-bisphosphate aldolase based on the previously described. In addition to that, *C. albicans,* an opportunistic pathogen, are selected as a model organism because its ability to differentiate from budding yeast to hyphae (Odds *et al*., 1985). With the unique characteristic, morphological change can be easily observed without difficulties.

Since *C. albicans* has been shown to be a diploid organism (Riggsby *et al*., 1982), sequential disruptions of apparent two copies of the gene were required for total elimination of expression and enzymatic activity of FBA1. *FBA1* heterozygous mutant (*fba1/FBA1*) was made as described previously and showed a large difference in *FBA1* expression levels (Fig. 4). However, *FBA1* null mutant could not be obtained. There has been reported that in *Saccharomyces cerevisiae,* haploid cells containing the defective aldolase allele lacks aldolase enzymatic activity and fail to grow on media containing a carbon source (Schwelberger *et al*., 1989). This suggests that it might be the fact that FBA1 is a critical enzyme that is necessary for cells to survive in *C. albicans*.

FBA1 overexpression mutant (KU104) was made (Fig. 5 and 6) and observed morphology. Interestingly, in YPD medium agar plate, SC5314 (wild-type) and

KU103 (CAI4-ADHpt) did not differentiate, but KU104 displayed emerging filament at 28 °C and formed little hyphae on the border of ovoid at 37 °C (Fig. 7 and 8). On the other hand, in spider medium agar plate, SC5314 and KU103 differentiated while KU104 did not (Fig. 9 and 10). In addition to that, cell morphology of KU104 consistent with the colony morphology (Fig. 11 and 12). Furthermore, by northern blot analysis, the expression of different differentiation genes was detected to confirm (Fig. 13 and 14). From these results, it was suspected that FBA1 might be responsible for increasing intracellular MG concentration to change morphology in KU104 since MG is known to be a toxic compound that causes DNA and protein damage both in vivo and in vitro in mammalian cells (Thornalley, 2008; Ramasamy *et al*., 2006).

To confirm the relationship between MG and FBA1, the intracellular MG concentration was measured using HPLC as described previously. As expected, the intracellular MG concentration of KU104 was increased in YPD medium and decreased in spider medium compared to SC5314 and KU103 (Fig. 15). These data demonstrate that FBA1 overexpression caused intracellular concentration MG to increase. Moreover, from these data, the increased level of reactive oxygen species (ROS) in KU104 was expected in YPD medium. Suzuki *et al*. reported that MG production strongly correlates with the generation of ROS including superoxide radicals during cell growth (2001).

To confirm the correlation between MG and ROS, the intracellular ROS was measured using a fluorescence spectrophotometer. As expected, the intracellular ROS level of KU104 increased in YPD medium and decreased in spider medium compared to SC5314 and KU103 (Fig. 17). These data confirmed that in KU104, both intracellular ROS and MG concentration increased in YPD medium. It suggested that differentiation in KU104 might be due to the intracellular MG concentration in glucose containing media. However, in general, mannitol containing media, intracellular concentration of MG increased. In fact, there might

be another enzyme, which relates to the production of MG during mannitol degradation pathway (Scheme 3). Also, when mannitol eventually degraded into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, there might be a chance that glyceraldehyde-3-phosphate form in L-form rather than D-form. In other word, L-glyceraldehyde-3-phosphate form and could not be used in a metabolism.

Choi *et al*. reported that MG accumulation by glutathione (GSH) depletion leads to cell cycle arrest in *Dictyostelium* (2008). To investigate whether the intracellular MG accumulation in KU104 leads to cell cycle arrest, the measurement of intracellular GSH and cell cycle analysis had been performed. As shown in Fig. 16, the intracellular GSH in KU104 was significantly lower in YPD medium and much greater in spider medium compared to SC5314 and KU103. In addition to that, cell cycle analysis displayed G1-phase arrest in KU104 (Fig. 18). In mammalian, the differentiation of cells precedes growth arrest in G1 phase (Scott *et al*., 1982). Taken together, these data suggest that FBA1 overexpression leads to MG accumulation and GSH declination that lead to G1-phase arrest.

In the present study, it demonstrated that overexpression of FBA1 or the accumulation of intracellular MG concentration might lead to differentiation in *C. albicans*. Further investigations about the relationship between MG and differentiation features in *C. albicans* are required.

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국문초록

Fructose-1,6-bisphosphate aldolase 는 해당 과정에서 중요하다고 알려져 있는 효소로써, fructose-1,6-bisphosphate 를 glyceraldehyde-3-phosphate 와 dihydroxyacetone phosphate 로 cleaveage 한다. 캔디다 알비칸스에서 fructose-1,6-bisphosphate aldolase (FBA1)를 인코딩하는 *FBA1* 을 클로닝 하였다. 캔디다 알비칸스의 *FBA1* 은 1080 개의 염기쌍으로 360 개의 아미노산으로 되어 있으며, 39,215 달톤의 분자량을 가진다. 캔디다 알비칸스에서 FBA1 의 세포내 역할을 관찰하기 위하여 *FBA1* 유전자를 결손 시키거나 과량 발현시켰다. 또한 *fba1/fba1* 동형접합형 돌연변이 균주를 제작하고자 하였으나 이 과정을 통해 선택한 돌연변이 균주는 생장을 확인하기 불가능하였다. 이 관찰을 통하여, *FBA1* 은 캔디다내에서 필수 유전자인 것으로 추측된다. 이형접합의 돌연변이 균주 (*FBA1/fba1)*는 야생형 (*FBA/FBA1)* 보다 현저하게 *FBA1* 발현량이 감소됨을 관찰하였다. *FBA1* 과량 발현 돌연변이균주에서는 야생형과 다른 표현형을 나타내었다: 글루코스를 탄소 공급원으로 하였을 때 *FBA1* 과량발현 균주는 hyphae 로 분화가 일어나며, 이를 보다 정확히 확인하기 위해 표지 유전자를 이용하여 노던법을 수행한 결과, true-hyphae 의 특정 유전자인 *HWP1* 이 발현됨을 확인하였다. 또한 캔디다에서 분화 배지로 알려진 마니톨 배지에서는 분화 경향을 전혀 나타내지 않았다. 더불어 *FBA1* 과량발현 돌연변이 균주의 세포내 메틸글리옥살의 양과 활성산소족의 농도가 유의적으로 증가하였고 글루타치온의 세포내 농도는 감소하였다. 이것은 *FBA1* 의

과 발현되어 메틸글리옥살의 양이 세포내에 축적했다는 것이다. 결론적으로, *FBA1* 과량 발현된 돌연변이 균주는 클루코스를 탄소 공급원으로 하는 배지에서 세포내 메틸글리옥살의 축적으로 인하여 G1 arrest 의 세포주기를 유도하며, *HWP1* 이 발현되어 true hyphae 로 분화를 촉진하는 것으로 보인다. 그러므로, fructose-1,6-bisphosphate aldolase 나 메틸글리옥살의 축적이 캔디다 알비칸스의 분화를 유도한다고 생각된다.

핵심어: 캔디다 알비칸스, fructose-1,6-bisphosphate aldolase, 메틸글리옥살, 분화

Roles of fructose-1,6-bisphosphate aldolase in *Candida albicans*

by

MyungHee Ku

Advisor:

Professor Sa-Ouk Kang, Ph.D.

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School of Biological Sciences

Graduate School

Seoul National University