Title

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Urea enhances cell lysis of fission yeast

Urea enhances cell lysis of *Schizosaccharomyces pombe ura4* mutants

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Abstract

Cell lysis is induced in *Schizosaccharomyces pombe* Δ*ura4* cells grown in YPD medium, which contains yeast extract, polypeptone, and glucose. To identify the medium components that induce cell lysis, we first tested various kinds of yeast extracts from different suppliers. Cell lysis of Δ*ura4* cells on YE medium was observed when yeast extracts from OXOID, BD, Oriental, and Difco were used, but not when using yeast extract from Kyokuto. To determine which compounds induced cell lysis, we subjected yeast extract and polypeptone to GC-MS analysis. Ten kinds of compounds were detected in OXOID and BD yeast extracts, but not in Kyokuto yeast extract. Among them was urea, which was also present in polypeptone, and it clearly induced cell lysis. Deletion of the *ure2* gene, which is responsible for utilizing urea, abolished the lytic effect of urea. The effect of urea was suppressed by deletion of *pub1*, and a similar phenotype was observed in the presence of polypeptone. Thus, urea is an inducer of cell lysis in *S. pombe* Δ*ura4* cells.

Key words: cell lysis; urea; fission yeast; UMP de novo synthesis; *ura4*

Introduction

Media composition is an important factor in phenotypic analyses of yeasts. *Saccharomyces cerevisiae* and closely related species are generally grown in YPD medium, which contains yeast extract, polypeptone, and dextrose (glucose). Meanwhile, *Schizosaccharomyces pombe* is usually grown on YE, which contains yeast extract, and glucose, but not polypeptone [1, 2]. Polypeptone, a protease degradation product of casein protein, is used as an amino acid and nitrogen source in YPD media, but has a deleterious effect on growth of *S. pombe*[3]. Yeast extract is sufficient to support growth when a carbon source is supplied. However, the composition of polypeptone and yeast extracts varies by producer, or even by lot from the same supplier. Growth of auxotrophic strains requires supplementation of media with nucleosides and amino acids. Auxotrophic markers are used for plasmid transformation, gene deletion, or diploidization, but auxotrophy can also affect phenotype: in *S. cerevisiae*, amino acid, inositol, and uracil concentrations influence growth of BY family strains [4, 5], whereas in *S. pombe*, G418 can be used to select on YE or EMM containing glutamate, but not on EMM medium containing ammonium chloride as nitrogen source [2]. Thus, the relationship between auxotrophic strains and compounds in the medium must be considered carefully.

The fission yeast *S. pombe* is a eukaryotic model organism used to study a wide range of molecular and cellular biological processes, including cell cycle regulation, signal transduction, transcription, chromatin structure, cell wall synthesis, and biotechnological applications [3, 6-11]. We found that cell lysis was induced in *S. pombe* Δ*ura4* cells by addition of polypeptone or depletion of uracil in the media [12]. Deletion of either one of *ura* genes (*ura1*, *ura2*, *ura3* and *ura5*) suppressed cell lysis of *Δura4* cells. Orotidine-5-monophosphate (OMP), a key compound in cell lysis, did not accumulate in these cells[12]. Furthermore, we found that cell lysis of Δ*ura4* cells was suppressed by addition of uracil or deletion of the *pub1* gene, which encodes an E3 ubiquitin ligase involved in the regulation of the *S. pombe* uracil transporter Fur4 [13]. Deletion of the *pub1* gene increased membrane localization of Fur4 protein, thereby increasing uracil uptake ability[13]. Thus, intracellular uracil concentration is critical for

cell lysis of Δ*ura4* cells. Uracil is used for synthesis of uridine-5-monophosphate (UMP) by the salvage pathway. Alternatively, UMP is also synthesized by the *de novo* pathway comprising Ura1, Ura2, Ura3, Ura4, and Ura5 (Fig. S1).

In this study, we investigated the relationship between cell lysis of *S. pombe* Δ*ura4* cells and components in yeast extract or polypeptone. Uracil concentration differed among five different yeast extracts and correlated with the cell lysis phenotype. GC-MS analysis identified 172 peaks in yeast extracts and polypeptone; among them, we determined that urea is an inducer of cell lysis in Δ*ura4* cells.

Materials and methods

Strains and media

The *S. pombe* strains used in this study are listed in Table 1. Standard yeast culture media and genetic manipulations were used. *S. pombe* strains were grown in complete YES medium (0.5% yeast extract, 3% glucose, and 225 mg/L each of adenine, leucine, uracil, histidine, and lysine hydrochloride) [1)], YE (1% yeast extract and 2% glucose), or YPD [1% yeast extract, 2% glucose, and 2% polypeptone (Nihon Pharmaceuticals Co. Ltd.)]. We used several different yeast extracts (Kyokuto Pharma. Ind., Co., Ltd; OXOID Ltd., BD & Co.; and Oriental Yeast Co., Ltd.) for comparison. Yeast extract from Kyokuto was used unless otherwise indicated. *S. pombe* strains were also grown in EMM medium (0.3% potassium hydrogen phthalate, 0.56% sodium phosphate, 0.5% ammonium chloride, 2% glucose, vitamins, minerals, and salts) [1)]. The appropriate auxotrophic supplements were added as necessary (10 or 225mg/l of uracil) to EMM. Polypeptone was added to a final concentration of 1 or 2%. Urea was added to a final concentration of 0.05, 0.1 or 0.4%.

Gene disruption

The chromosomal *ure2* gene was disrupted by homologous recombination using PCR-generated fragments [14)]. The 1.2 kb *natMX6* modules were amplified with flanking homology sequences of the *ure2* gene[15)] using primers listed in Table S1. Correct

disruption of the gene of interest was verified by colony PCR using the appropriate primers [16].

Spotted and BCIP assay

Cells were pre-cultured in YES liquid medium for 12 h at 30°C, and then re-suspended in water at a density of 2×10^6 cells/mL. Cell suspensions were serially diluted (1:10) and plated on YES, YPD, YE, or YE+urea plates and incubated for 3 days at 30°C. For the alkaline phosphatase assay, each plate was overlaid for 10, 30, or 60 min with a phosphatase assay solution containing 0.05 M glycine-NaOH (pH9.8), 1% agar, and 2.5 mg/mL 5-bromo-4-chloro-3-indolylphosphate (BCIP).

Medium sample preparation for GC-MS

Samples from media were prepared by adding 900 µL of extract buffer (methanol:chloroform:water = 5:2:2) to 100 µL of medium, and proteins were removed by centrifugation (14,000 rpm, 4°C, 10 min). Then, 600 µL of supernatant was transferred to a new tube, dried in a centrifugal evaporator, and freeze-dried. For oximation, 80 µL of methoxyamine hydrochloride in pyridine (20 mg/ml) was added, and the sample was incubated at 30°C for 90 min. For trimethylsilylation, 40 µL of *N*-methyl-*N*-(trimethylsilyl) trifluoroaceramide (MSTFA) was added, and the sample was incubated at 37°C for 30 min.

Uracil concentration using stable isotopic uracil

Samples from media were prepared by adding 900 µL of extract buffer (methanol:chloroform:water = 5:2:2) to 100 µL of medium, and proteins were removed by centrifugation (14,000 rpm, 4°C, 10min). Supernatant (600 µL) was transferred to a new tube and dried on a centrifugal evaporator. Next, 150 µL of 50% acetonitrile was added to the dry sample and diluted 10-fold. Then, an equal amount of 0.5 ppm ^{13}C uracil was added to the diluted sample. LC-MS data were obtained using a MassLynx system (Waters) coupled to a Xevo-TQ mass spectrometer (Waters). LC separation was performed on an ACQUITY UPLC BEH Amide column (Merck SeQuant; 2.1×100 mm,

1.7 μm particle size). Buffer A (acetonitrile + 0.1% formic acid) and buffer B (H_2O + 0.1% formic acid) were used as the mobile phase, with gradient elution from 99% A (1% B) to 30% A (80% B) in 7 min at a flow rate of 0.4 mL/min. The initial conditions were restored after 10 min and maintained for 5 min at a flow rate of 0.4 mL/min. ^{12}C Uracil was detected using MRM mode [ESI(+) 113>96], and ^{13}C uracil was detected using MRM mode [ESI(+) 114>97].

Urea measurement

Urea assay Kit (BioAssay Systems) was used. 50 μL samples, water (blank), and standard urea (5 mg/dL) in duplicate were transferred into wells of a clear bottom 96-well plate. 200 μL working reagent was added and tapped lightly to mix. Samples were incubated 50 min at room temperature and optical density at 430nm was measured.

Reproducibility

All experiments were conducted at least twice to confirm the reproducibility of the results.

Results

Polypeptone supported growth of uracil auxotrophic strains

To better understand the role of polypeptone on the inducing effect of cell lysis of *S. pombe ura4* gene mutants [12], we first tested the effect of polypeptone on the growth of uracil auxotrophic strains. We inoculated five uracil auxotrophic strains (*Δura1*, *Δura2*, *Δura3*, *Δura4*, and *Δura5* strains), all of which are defective in *de novo* UMP synthesis, onto various media and incubated for 3 days at 30°C (Fig. 1A). None of the strains grew without uracil (Fig. 1A; comparison of second and third panels), but polypeptone restored growth without additional uracil [Fig. 1A, fourth panel; grown on PG media (2% polypeptone and 2% glucose)], indicating that polypeptone contains uracil (or compounds related to uracil) for the synthesis of UMP. Polypeptone is generally used as

a source for amino acids or nitrogen for culture medium, but our result indicates that it apparently contains other ingredients that support UMP synthesis. We next tested the growth of uracil auxotrophic strains on EMM+polypeptone medium (fifth panel). None of the uracil auxotrophic strains grew well on this medium, but they grew much better on EMM+polypeptone in the absence of ammonium salt as a nitrogen source (sixth panel), indicating that excess nitrogen inhibits their growth under this condition. Although uracil auxotrophic strains grew on EMM medium containing a low amount of uracil (10 mg/mL), the growth of uracil auxotrophic strains was retarded by the addition of 2% polypeptone (Fig. 1B). These results indicate that excess nitrogen source, such as polypeptone, in the medium inhibits the growth of uracil auxotrophic strains. Thus, while polypeptone supported the growth of uracil auxotrophic strains, it also inhibited growth under different conditions. These results are consistent with a previous study showing that growth of *ura4* deletion mutants is inhibited by 5 g/L NH_4Cl in YE medium [17].

Uracil concentration in yeast extracts are different.

We previously showed that Δ*ura4* cells underwent cell lysis in YE medium, which does not contain polypeptone [12], but this result was not always reproducible [13]. We suspected that this difference was caused by differences in the yeast extract used in our previous two experiments. We then tested various kinds of yeast extracts made by different suppliers. Cell lysis of Δ*ura4* cells on YE medium was observed when we used yeast extracts from BD, OXOID, Oriental, and Difco, but not when we used Kyokuto yeast extract (Fig. 2A). Cell lysis was observed when polypeptone was included in any media. Deletion of the *fur4* gene, which encodes a uracil transporter, in Δ*ura4* cells induced cell lysis even in medium containing Kyokuto yeast extract. These results indicate that components of yeast extract differ among suppliers. Because we knew that uracil suppressed cell lysis of Δ*ura4* cells when it was grown on YE medium [12, 13], we hypothesized that these differences were related to a difference in the uracil concentration among yeast extracts. We then measured the uracil concentration of five yeast extracts by LC-MS/MS, using the stable isotope ^{13}C-uracil as an internal standard. Indeed, the uracil concentration of Kyokuto yeast extract was two to four times higher

than those of the four other yeast extracts (Fig. 2B). By contrast, Difco yeast extract contained the lowest uracil concentration. *Δura4* cells did not lyse in YE (Difco) media when more than 20 mg/L uracil was added (Fig. 2C). We also tested the effect of high concentrations of yeast extract in YE media (4% yeast extract and 2% glucose), expecting that increasing the uracil concentration would suppress cell lysis. However, Δ*ura4* cells lysed in media containing 4% yeast extract (Kyokuto, OXOID, BD, oriental, and Difco) (Fig. 2D). These results indicate that uracil is a factor that can reverse cell lysis of Δ*ura4* cells, but is not the sole determinant of lysis, suggesting that there are other factors in yeast extract that affect cell lysis.

GC-MS analysis of yeast extract

The results described above suggested that yeast extract and polypeptone contain unidentified compounds that induce cell lysis of Δ*ura4* cells. To identify these compounds, we subjected yeast extract and polypeptone to GC-MS (Shimadzu GC-MS 2010 ultra) analysis after treatment with TMS (trimethylsilylation). Raw data were analyzed using the Smart Metabolites Database system. In our analysis, we detected about 172 peaks from yeast extract and polypeptone (all compounds are indicated in Table S2). Uracil concentration in Kyokuto yeast extract was 2.2–2.9 times higher than those of the OXOID and BD yeast extracts, respectively (Table 2), consistent with the LC-MS/MS data (Fig. 2B). Based on this result, we believe that the GC-MS data are reliable. Nine kinds of compounds detected in GC-MS analysis had higher peak areas in Kyokuto yeast extract than in OXOID and BD yeast extracts (Table 2). On the other hand, ten kinds of compounds were detected in OXOID and BD yeast extracts, but not in Kyokuto yeast extract (Table 3). Furthermore, 27 compounds were specifically detected in polypeptone (Table 4). Among these 27 compounds, 14 were sugars, but the isomeric structures of these compounds could not be determined. We speculate that these compounds are potentially related to cell lysis of Δ*ura4* cells, either as suppressors or inducers.

Urea induces cell lysis of Δ*ura4* cells

Based on the results described above, we next tested the compounds detected in polypeptone for their ability to induce cell lysis. For this purpose, we made YE medium containing seven different sugars or urea, and spotted Δ*ura4* cells on the various media. None of the sugars we tested (sucrose, maltose, lactose, galactose, mannose, mannitol, and xylose) significantly affected the growth and lysis of Δ*ura4* or Δ*ura4* Δ*fur4* cells (11). However, cell lysis in Δ*ura4* and Δ*ura4* Δ*fur4* cells was induced when they were grown on YE containing 0.1% urea (Fig. 3A). Because 1% inhibited the growth of all tested strains, we used 0.1% urea for subsequent experiments. We also noticed that deletion of *fur4* increased sensitivity to urea (Fig. 3A and B). We then spotted Δ*ura4* cells onto YE containing different amount of urea (0%, 0.1%, 0.15%, and 0.2%). Cell lysis of Δ*ura4* cells was induced on YE containing urea (0.1%, 0.15%, and 0.2%) but suppressed by supplementation with uracil (300 mg/L) (Fig. 3B). Moreover, deletion of the *pub1* gene, which encodes E3 ubiquitin ligase, or the *ura5* gene, which encodes orotate phosphoribosyltransferase, strongly suppressed cell lysis of Δ*ura4* cells on YE containing urea. Because deletion of Pub1 enhances uracil incorporation[13], it antagonizes the role of urea. Deletion of *ura5* in Δ*ura4* cells suppressed cell lysis by urea, as observed in YPD, because orotidine-5-monophasphate (OMP), a key compound in cell lysis, does not accumulate in Δ*ura5* Δ*ura4* cells. Because polypeptone inhibited growth of uracil auxotrophic strains in uracil-containing medium, as shown in Fig. 1, we asked whether a similar effect could be seen in medium containing urea. We spotted uracil auxotrophic (Δ*ura1*, Δ*ura2*, Δ*ura3*, Δ*ura4*, and Δ*ura5*) strains on low uracil-containing medium (10 mg/L) with or without urea. Growth of uracil auxotrophic strains was inhibited by urea (Fig. 3C). This growth inhibition of uracil auxotrophic strains by urea was suppressed by addition of a high concentration of uracil (Fig 3D). Cell lysis was clearly observed under the microscope by the addition of urea (Fig. 3E). We further examined the effect of uracil transporter Fur4 on the growth of EMM in the presence of polypeptone or urea. Δ*ura4* Δ*fur4* cells exhibited stronger sensitivity to polypeptone or urea than Δ*ura4* and Δ*fur4* cells (Fig. 3F). Thus, urea has an effect similar to that of polypeptone on cell lysis of Δ*ura4* cells and inhibition of growth on EMMU. However, when we measured concentration of urea in yeast extracts and polypeptone by urea assay kit, urea concentrations were about 0.0017-0.0022% in BD

yeast extract, OXOID yeast extract or polypeptone (Fig. S3). These concentrations of urea are too low to induce cell lysis.

Deletion of *ure2* suppressed cell lysis of Δ*ura4* cells grown on YE containing urea

We next tested the role of urea in cell lysis by deleting the *ure2* gene, which encodes an ATP-independent urease that catalyzes the formation of $2NH_3$ from urea[18]. (The *ure2* gene has been mapped [19] and shown to be identical to the *ure1* gene [20]). Urea can be used as a nitrogen source in *S. pombe* expressing the Ure2 protein[20]; consequently, Δ*ure2* cells cannot grow on medium containing urea (0.01 M) as the sole nitrogen source. We constructed Δ*ura4*Δ*ure2* double mutants to further examine the effect of *ure2* on the cell growth and cell lysis of Δ*ura4* cells. Deletion of the *ure2* gene suppressed the growth inhibitory effect of urea (Fig. 4A) and significantly suppressed cell lysis of Δ*ura4* cells on YE containing urea (Fig. 4B). However, Δ*ura4* Δ*ure2* cells still lysed on YPD medium, suggesting that polypeptone contains another inducible factor in addition to urea. Next, we investigated whether Δ*ura4* Δ*ure2* cells would lyse on YE (OXOID, BD, Oriental, or Difco) medium. Deletion of the *ure2* gene significantly suppressed cell lysis of Δ*ura4* cells on YE (OXOID) medium and weakly suppressed cell lysis of Δ*ura4* cells on YE (BD or Oriental) medium (Fig. 4C). However, deletion of the *ure2* gene in Δ*ura1*, Δ*ura2,* Δ*ura3,* Δ*ura4* or Δ*ura5* cells did not suppress growth inhibition by urea in low uracil-containing medium (10mg/mL) (Fig. S4A).

Discussion

The components of yeast extract affect the cell lysis phenotype of *S. pombe* Δ*ura4.*

In this study, we sought to identify the medium components that induce cell lysis of Δ*ura4* cells in YPD media. We reported previously that polypeptone induces cell lysis of Δ*ura4* cells in YPD medium[12, 13], but as shown in this study, yeast extract also affected cell lysis (Fig. 2A). The main factor explaining why yeast extract affects cell lysis is the difference in the concentration of uracil among yeast extract from different suppliers (Fig. 2B). In light of our previous observation that depletion of uracil induced cell lysis, whereas supplementation of uracil suppressed cell lysis in YPD media [12], it is

reasonable to postulate that the amount of uracil in yeast extract affects the cell lysis phenotype. However, the uracil in yeast extract is not the sole determinant of the cell lysis-inducing effect. Increasing the amount of yeast extract to 4% did not reverse the effect in Δ*ura4* cells (Fig. 2C and D), indicating that some factors (such as urea) antagonize the increased uracil amount by increasing yeast extract.

We measured compounds in yeast extract and polypeptone using GC-MS, and identified 172 peaks in total. Nine kinds of compounds detected by GC-MS analysis were more abundant in Kyokuto yeast extract than in OXOID and BD yeast extracts (Table 2), whereas ten peaks were detected in BD and OXOID but not in Kyokuto yeast extract (Table 3). We added some of the products identified by GC-MS to the media, and tested cell lysis by BCIP assay (Fig. S2). The seven sugars tested did not affect cell lysis. However, urea, which was not detected in the Kyokuto yeast extract, clearly induced cell lysis in Δ*ura4* cells (Fig. 3A). Urea was also detected in polypeptone (Table S2, Fig. S3) and had an effect similar to that of polypeptone, as addition of uracil suppressed cell lysis in YE containing urea. In addition, the cell lysis of Δ*ura4* cells in YE containing urea was suppressed by deletion of *pub1*, which encodes an E3 ligase that regulates uracil transporter, or *ura5*, which encodes orotate phosphoribosyltransferase (Fig. S1). We concluded that urea is one of the compounds that induces cell lysis of Δ*ura4* cells, and that the balance between suppression of cell lysis by uracil and induction of lysis by urea determines the overall extent of cell lysis. Even though we observed 0.1 % urea induced cell lysis, the concentrations of urea included in yeast extracts and polypeptone were very low (about 0.002 %). This indicates urea is not a sole determinant in inducing cell lysis and suggests that other nitrogen sources such as peptides or amino acids together can induce cell lysis in Δ*ura4* cells.

Urea induces cell lysis of Δ*ura4* cells by increasing the intracellular NH_3 concentration

Deletion of the *ure2* gene, which encodes an ATP-independent urease [20, 21], suppressed cell lysis of Δ*ura4* cells in YE+urea media. However, deletion of the *ure2* gene did not clearly suppress cell lysis of Δ*ura4* cells in YPD media (Fig. 4A and B). Therefore, we

317 believe that urea does not directly affect cell lysis of *Δura4* cells, but instead induces
318 lysis by increasing the intracellular concentration of ammonia (NH_3), which is
319 generated through hydrolysis of urea by urease. Because polypeptone contains low
320 molecular weight peptides and various amino acids, these are converted to NH_3. Thus,
321 intracellular NH_3 may be the inducer of cell lysis resulting from treatment with urea or
322 various amino acids (Fig. 5).
323
324 **Excess nitrogen source may inhibit transport of uracil or the UMP salvage**
325 **pathway**
326 Based on our results, we hypothesize that excess nitrogen inhibits uracil transport
327 and/or the UMP salvage pathway, for the following reasons. 1) Uracil auxotrophic
328 strains, all defective in the *de novo* UMP synthetic pathway, exhibited growth
329 retardation by addition of polypeptone in EMM (Fig. 1A). 2) Growth of uracil
330 auxotrophic strains was inhibited by polypeptone or urea on EMM medium containing
331 low concentrations of uracil (Fig. 1B and 3C). 3) Growth of Δ*ura4* mutants on YE
332 media was sensitive to addition of NH_4Cl or urea. 4) Δ*ura4* Δ*fur4* cells were more
333 sensitive to polypeptone or urea on EMMU media than Δ*ura4* cells. 5) Cell lysis was
334 induced in Δ*ura4* Δ*fur4* cells on YE containing urea (0.1%) (Fig. 3B and E). All of these
335 results can be explained if nitrogen, probably NH_3, inhibits the transport of uracil and/or
336 the salvage pathway of UMP synthesis. Uracil in the media is necessary for transport,
337 mainly (but not solely) through Fur4, into the cell, where it is converted to UMP by the
338 salvage pathway. Thus, transport of uracil and the UMP salvage pathway are necessary
339 for growth of uracil auxotrophic strains. We previously found that localization of Fur4
340 was regulated by a nitrogen source in media[13]. However, because Δ*ura4* Δ*fur4* cells
341 were more sensitive to polypeptone (or urea) than Δ*ura4* cells when grown on EMMU
342 medium and they grew better without polypeptone or urea (Fig. 3F), our results suggest
343 that excess nitrogen source is also inhibitory to UMP salvage pathway. If the transporter
344 (Fur4) is the sole target of polypeptone or urea, no such phenotype should be observed.
345 At present, we do not know whether regulation of the transporter or the UMP salvage
346 pathway is more relevant to the mechanism by which excess nitrogen source affects
347 uracil auxotrophic strains. However, the phenotypes we observed are closely related to

the availability of uracil inside the cell.

Excess NH_4Cl inhibits growth of the Δ*leu1* or Δ*eca39* mutants in YE media [17, 22]. This phenotype can be suppressed by nitrogen signaling factors (NSFs) or supernatant from a prototrophic strain[19]. However, the growth of *ade6* and *ura4* single mutants was not promoted by these conditions. Thus, the mechanism of sensitivity to excess nitrogen differs between *leu1* and *ura4* mutants, but apparently excess nitrogen affects cell growth. Excess NH_4Cl did not inhibit growth of uracil auxotrophic strains and not induce cell lysis of Δ*ura4* cells in low uracil-containing EMM medium (Fig. S4B). We think that the effect of NH_4Cl in EMM media is different from YE media and that other compounds in yeast extract affect the phenotype.

In conclusion, we found in this study that among the components included in polypeptone and yeast extracts, uracil is a suppressor and urea is an inducer of cell lysis in *S. pombe* Δ*ura4* cells. To be effective, urea must be converted into ammonia, and excess intracellular nitrogen somehow inhibits the availability of uracil inside cells. Thus, uracil depletion induces cell lysis of *S. pombe* Δ*ura4*.

Author contributions

K.N. performed the experiments and wrote the manuscript. Mi.K. performed the experiments. Y.M. and T.K. provided technical assistance and advice. Ma.K. designed the experiments, analyzed the data, and wrote the manuscript.

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453 **Figure legends**

454 Fig. 1. Excess nitrogen source inhibits growth of uracil auxotrophic strains. (A) L972
455 (WT; *ura4*$^{+}$), UMP34 (Δ*ura1*), UMP35 (Δ*ura2*), UMP36 (Δ*ura3*), UMP31 (Δ*ura4*), and
456 UMP37 (Δ*ura5*) were pre-cultured for 12 h, and then spotted onto YES, EMM, EMMU
457 (225 mg/L uracil), PG (Polypeptone 2% and Glucose 2%), EMM+Polypeptone, or
458 EMM (-NH_4Cl)+Polypeptone media, and incubated for 3 days at 30°C. (B) The same
459 strains were pre-cultured for 12 h, and then spotted onto YES, EMM, and EMMU (10
460 mg/L uracil) in the presence or absence of polypeptone (0%, 1%, or 2%) and incubated
461 for 3 days at 30°C.

462

463 Fig. 2. Uracil is an important determinant of cell lysis of Δ*ura4* strains in YE medium.
464 (A) L972 (WT; *ura4*$^{+}$), UMP31 (Δ*ura4*), KNP16 (Δ*fur4*), and KNP27 (Δ*ura4* Δ*fur4*)
465 were grown for 12 h, spotted onto YE medium containing yeast extract from the
466 indicated supplier (Kyokuto, BD, OXOID, Oriental Yeast, or Difco) in the presence or
467 absence of polypeptone (2%), and incubated for 3 days at 30°C. For the alkaline
468 phosphatase assay, the plates were overlaid for 60 min with a phosphatase assay
469 solution containing 50 mM glycine-NaOH (pH 9.8), 1% agar, and 2.5 mg/mL of BCIP.
470 (B) Uracil concentration of yeast extract from each supplier, measured by LC-MS/MS.
471 (C) L972 (WT; *ura4*$^{+}$), UMP31 (Δ*ura4*), KNP16 (Δ*fur4*), and KNP27 (Δ*ura4* Δ*fur4*)
472 were grown for 12 h, spotted onto YE in the presence or absence of uracil (20, 30, 60,
473 or 70 mg/l), and incubated for 3 days. (D) L972 (WT; *ura4*$^{+}$) and UMP31 (Δ*ura4*) cells
474 were grown for 12 h, spotted onto YE medium (1% or 4% OXOID, BD, Oriental, or
475 Difco yeast extract), and incubated for 3 days. For the alkaline phosphatase assay, BCIP
476 was used as described in (A).

477 Fig. 3. Urea induces cell lysis of Δ*ura4* strains in YE medium. (A) L972 (WT; *ura4*$^{+}$),
478 UMP31 (Δ*ura4*), KNP27 (Δ*ura4* Δ*fur4*), UMP37 (Δ*ura5*), and PR109 (*ura4-D18*) were
479 grown for 12 h, spotted onto YE in the presence or absence of urea (0.05%, 0.1%, or
480 1%), and incubated for 3 days. For the alkaline phosphatase assay, BCIP was used as
481 described in Fig. 2. (B) L972 (WT; *ura4*$^{+}$), UMP31 (Δ*ura4*), KNP27 (Δ*ura4* Δ*fur4*),

UMP42 (Δ*ura4* Δ*ura5*), KNP32 (Δ*ura4* Δ*pub1*), and KNP38 (Δ*ura4* Δ*pub1* Δ*fur4*) were grown for 12 h, spotted onto YE in the presence or absence of urea (0.1%, 0.15%, or 0.2%) or uracil (300 mg/L), and incubated for 3 days. For the alkaline phosphatase assay, BCIP was used as described in Fig. 1. Cells were also observed by microscopy after 3 days of growth on YE+urea. (C) L972 (WT; *ura4*$^{+}$), UMP34 (Δ*ura1*), UMP35 (Δ*ura2*), UMP36 (Δ*ura3*), UMP31 (Δ*ura4*), and UMP37 (Δ*ura5*) were grown for 12 h, and then spotted onto EMMU (10mg/L uracil) in the presence or absence of urea (0%, 0.1%, or 0.4%) and incubated for 3 days. (D) L972 (WT; *ura4*$^{+}$), UMP34 (Δ*ura1*), UMP35 (Δ*ura2*), UMP36 (Δ*ura3*), UMP31 (Δ*ura4*), and UMP37 (Δ*ura5*) were grown for 12 h, and then spotted onto EMMU (10 or 225mg/L uracil) in the presence of urea (0.1%) and incubated for 3 days. (E) L972 (WT; *ura4*$^{+}$) and UMP31 (Δ*ura4*) were also observed by microscopy after 3 days on YE, YE+urea, or YPD. (F) L972 (WT; *ura4*$^{+}$), UMP31 (Δ*ura4*), KNP16 (Δ*fur4*), and KNP27 (Δ*ura4* Δ*fur4*) were grown for 12 h, spotted onto EMMU (200 mg/L uracil) in the presence or absence of polypeptone (0%, 0.5%, 1%, 2%, or 3%) or urea (0.05%, 1%, or 2%), and incubated for 4 days at 30°C.

Fig. 4. Deletion of the *ure2* gene suppresses cell lysis of Δ*ura4* on YE+urea medium. (A) L972 (WT; *ura4*$^{+}$), UMP31 (Δ*ura4*), KNP174♯1 (Δ*ure2*), KNP174♯2 (Δ*ure2*), KNP176♯1 (Δ*ura4* Δ*ure2*), and KNP176♯2 (Δ*ura4* Δ*ure2*) were grown for 12 h, spotted onto YE in the presence or absence of urea (0.01%, 0.1%, 0.15%, or 0.2%), and incubated for 3 days. Strains #1 and #2 are different isolates and are otherwise of the same genetic background. (B) For the alkaline phosphatase assay, BCIP was used as described in Fig. 1. (C) L972 (WT; *ura4*$^{+}$), UMP31 (Δ*ura4*), KNP174 (Δ*ure2*), and KNP176 (Δ*ura4* Δ*ure2*) were grown for 12 h, spotted onto YE medium (Kyokuto, BD, OXOID, Oriental Yeast, and Difco) in the presence or absence of polypeptone (2%), and incubated for 3 days at 30°C. For the alkaline phosphatase assay, BCIP was used as described in Fig. 1.

Fig. 5. A model for induction of cell lysis

In fission yeast, urease (Ure2) catalyzes the hydrolysis of urea to yield $2NH_3$ and CO_2. Polypeptone supplies amino acid or low molecular weight peptide, and therefore

512 increases the intracellular concentration of NH_3. The increase in intracellular
513 concentration of NH_3 is likely to inhibit uracil transport and/or the salvage pathway of
514 UMP synthesis. Depletion of intracellular uracil then causes cell lysis.
515

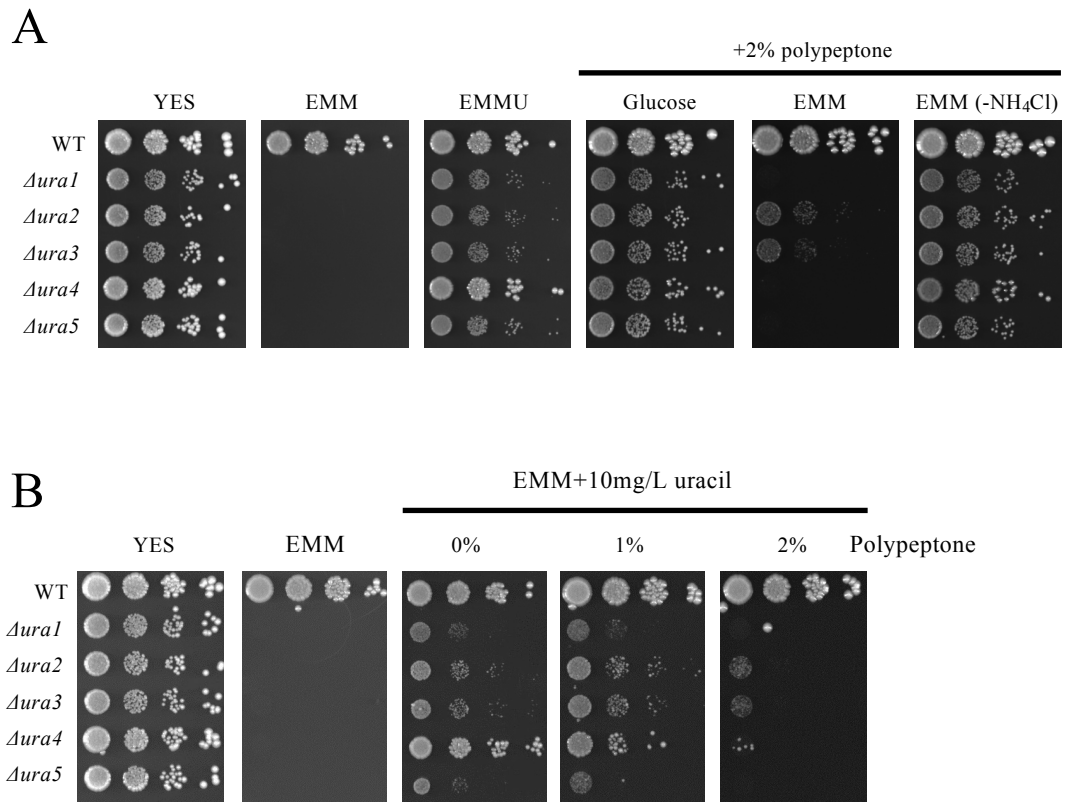
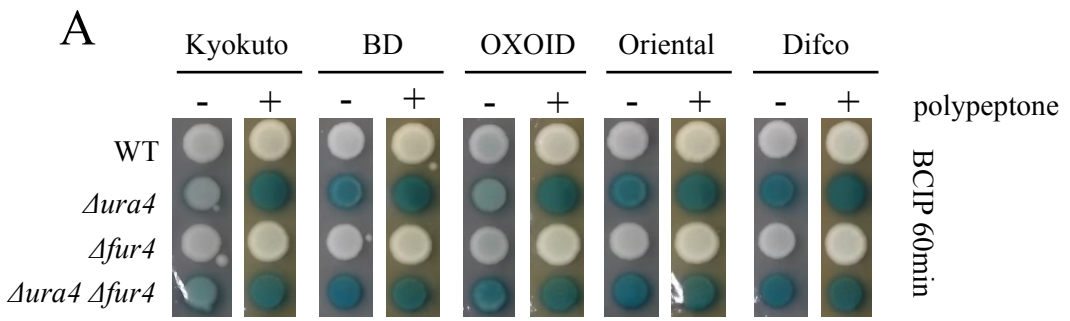
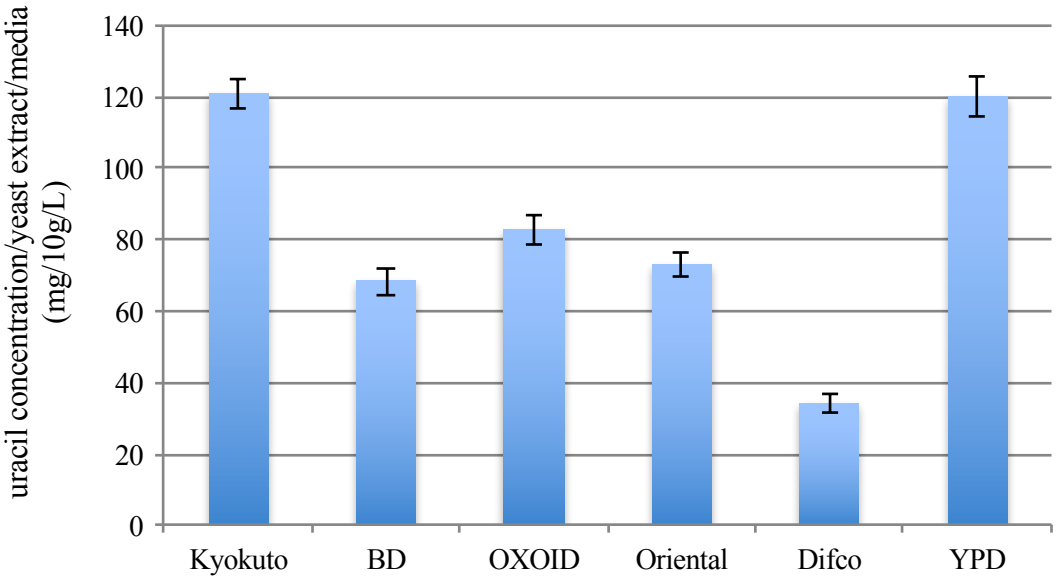
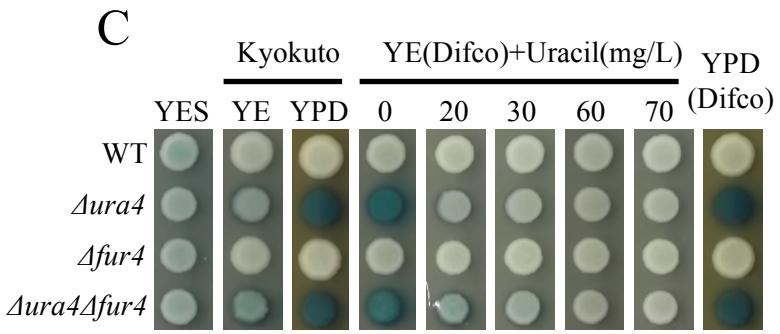

A
+2% polypeptone
YES
EMM
EMMU
Glucose
EMM
EMM (-NH4Cl)
WT
Δura1
Δura2
Δura3
Δura4
Δura5
B
EMM+10mg/L uracil
YES
EMM
0%
1%
2%
Polypeptone
WT
Δura1
Δura2
Δura3
Δura4
Δura5

Fig. 1


A
Kyokuto
BD
OXOID
Oriental
Difco
- + - + - + - + - +
polypeptone
BCIP 60min
WT
Δura4
Δfur4
Δura4 Δfur4


B
uracil concentration/yeast extract/media (mg/10g/L)
140
120
100
80
60
40
20
0
Kyokuto
BD
OXOID
Oriental
Difco
YPD


C
Kyokuto
YE(Difco)+Uracil(mg/L)
YPD (Difco)
YES
YE
YPD
0
20
30
60
70
WT
Δura4
Δfur4
Δura4Δfur4

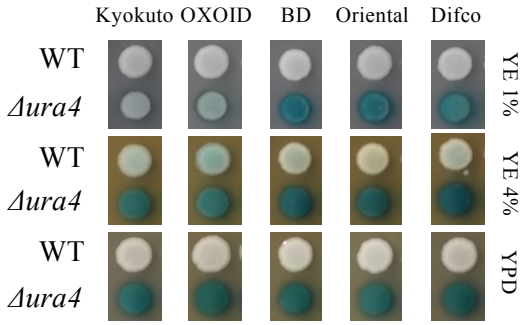

D
Kyokuto
OXOID
BD
Oriental
Difco
WT
Δura4
WT
Δura4
WT
Δura4
YE 1%
YE 4%
YPD

Fig 2

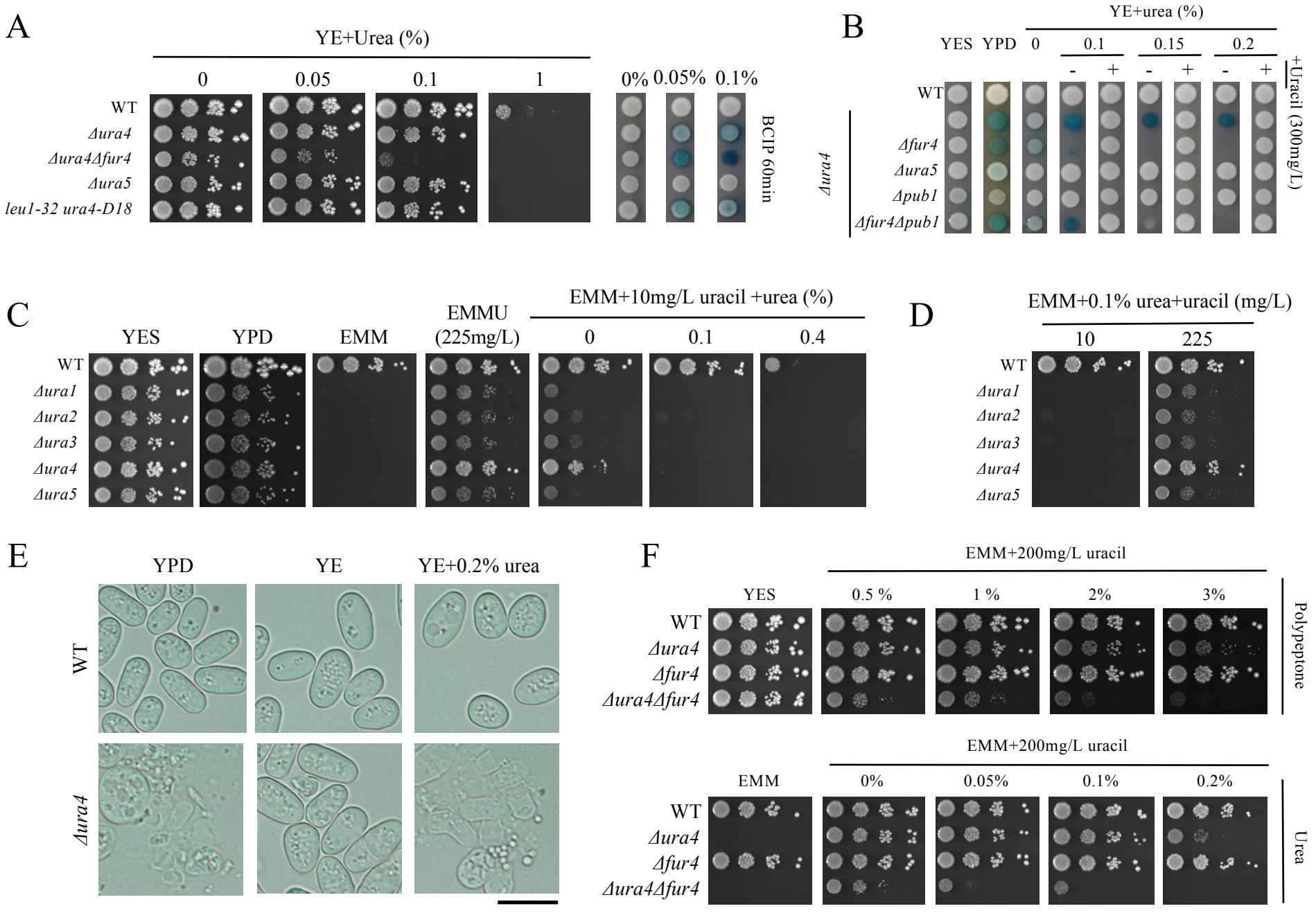

A
YE+Urea (%)
0
0.05
0.1
1
0%
0.05%
0.1%
WT
Δura4
Δura4Δfur4
Δura5
leu1-32 ura4-D18
BCIP 60min
B
YE+urea (%)
YES
YPD
0
0.1
0.15
0.2
-
+
-
+
-
+
+Uracil (300mg/L)
WT
Δura4
Δfur4
Δura5
Δpub1
Δfur4Δpub1
C
YES
YPD
EMM
EMMU (225mg/L)
EMM+10mg/L uracil +urea (%)
0
0.1
0.4
WT
Δura1
Δura2
Δura3
Δura4
Δura5
D
EMM+0.1% urea+uracil (mg/L)
10
225
WT
Δura1
Δura2
Δura3
Δura4
Δura5
E
YPD
YE
YE+0.2% urea
WT
Δura4
F
EMM+200mg/L uracil
YES
0.5 %
1 %
2%
3%
WT
Δura4
Δfur4
Δura4Δfur4
Polypeptone
EMM+200mg/L uracil
EMM
0%
0.05%
0.1%
0.2%
WT
Δura4
Δfur4
Δura4Δfur4
Urea

Fig 3

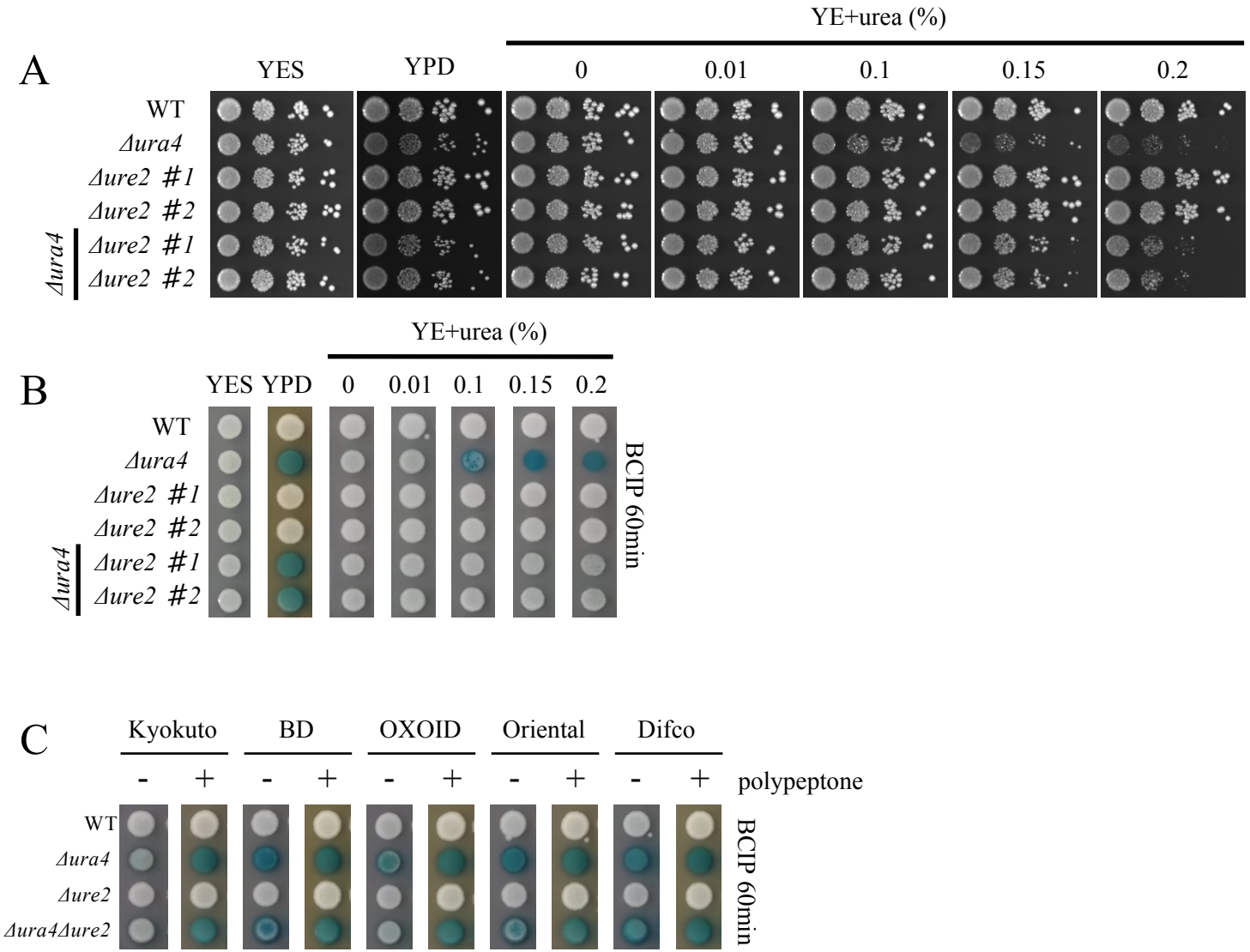

A
YE+urea (%)
YES
YPD
0
0.01
0.1
0.15
0.2
WT
Δura4
Δure2 #1
Δure2 #2
Δura4
Δure2 #1
Δure2 #2
B
YE+urea (%)
YES YPD
0
0.01
0.1
0.15
0.2
WT
Δura4
Δure2 #1
Δure2 #2
Δura4
Δure2 #1
Δure2 #2
BCIP 60min
C
Kyokuto
BD
OXOID
Oriental
Difco
- + - + - + - + - +
polypeptone
WT
Δura4
Δure2
Δura4Δure2
BCIP 60min

Fig 4

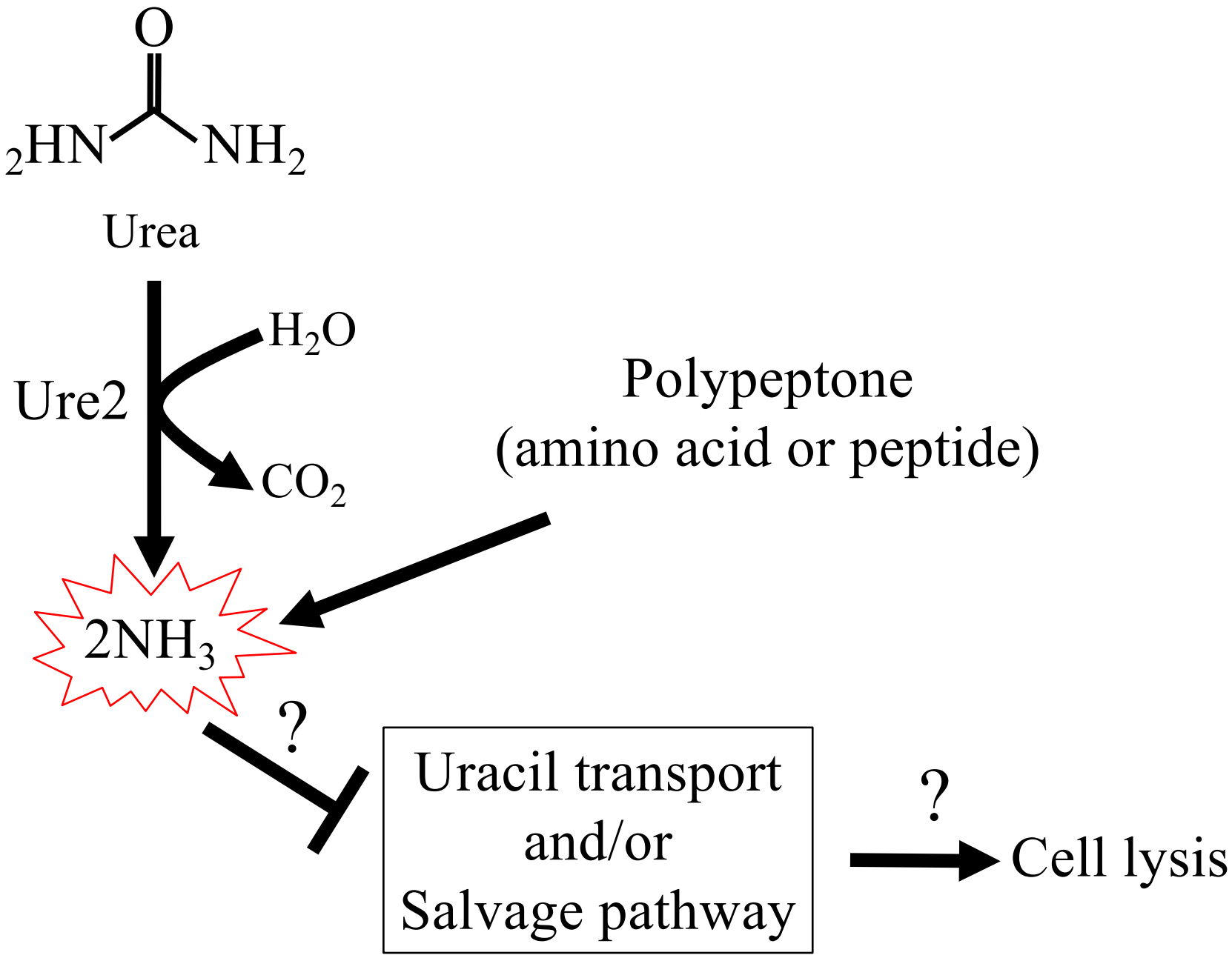

O
2HN
NH2
Urea
Ure2
H2O
CO2
Polypeptone
(amino acid or peptide)
2NH3
?
Uracil transport
and/or
Salvage pathway
?
Cell lysis

Fig 5

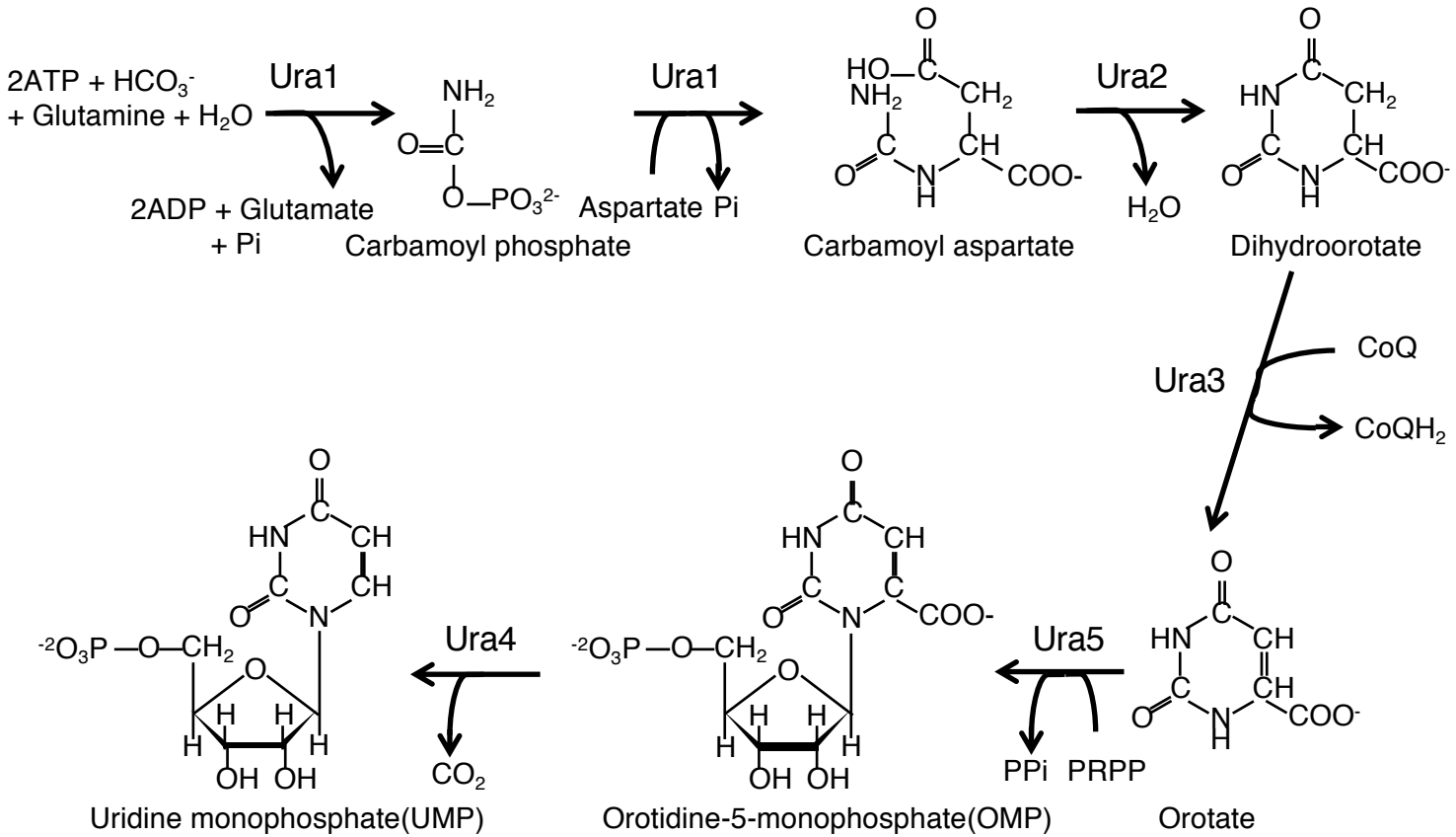

2ATP + HCO3-
+ Glutamine + H2O
Ura1
2ADP + Glutamate
+ Pi
Carbamoyl phosphate
Ura1
Aspartate Pi
Carbamoyl aspartate
Ura2
H2O
Dihydroorotate
Ura3
CoQ
CoQH2
Orotate
Ura5
PPi PRPP
Orotidine-5-monophosphate(OMP)
Ura4
CO2
Uridine monophosphate(UMP)

Fig S1

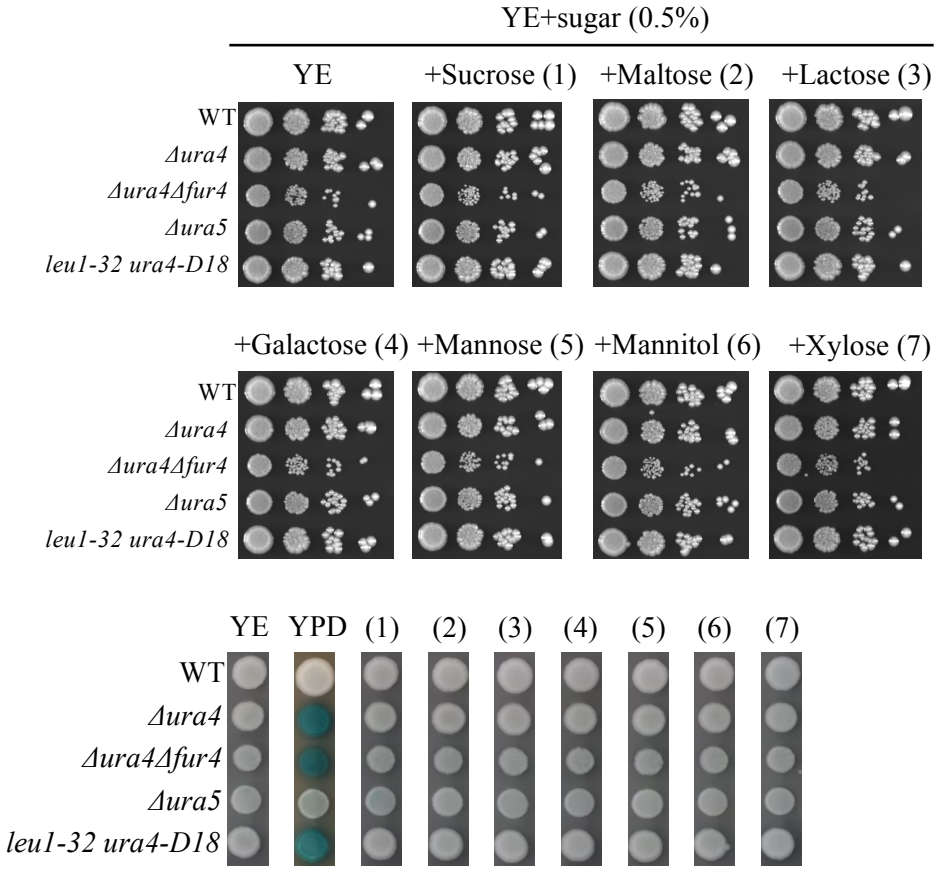

YE+sugar (0.5%)
YE
+Sucrose (1)
+Maltose (2)
+Lactose (3)
WT
Δura4
Δura4Δfur4
Δura5
leu1-32 ura4-D18
+Galactose (4)
+Mannose (5)
+Mannitol (6)
+Xylose (7)
WT
Δura4
Δura4Δfur4
Δura5
leu1-32 ura4-D18
YE
YPD
(1)
(2)
(3)
(4)
(5)
(6)
(7)
WT
Δura4
Δura4Δfur4
Δura5
leu1-32 ura4-D18

Fig S2

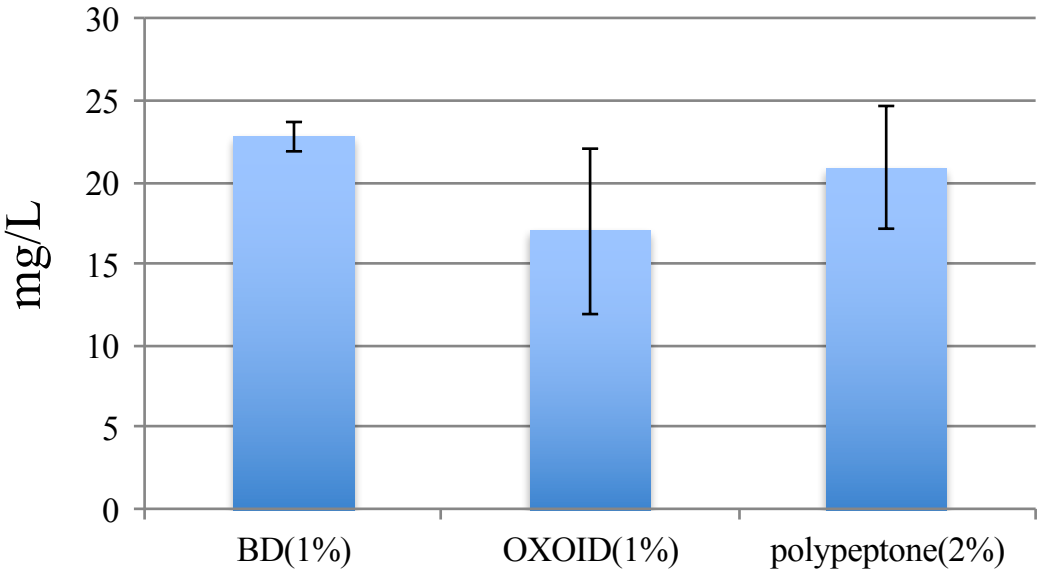


Fig S3

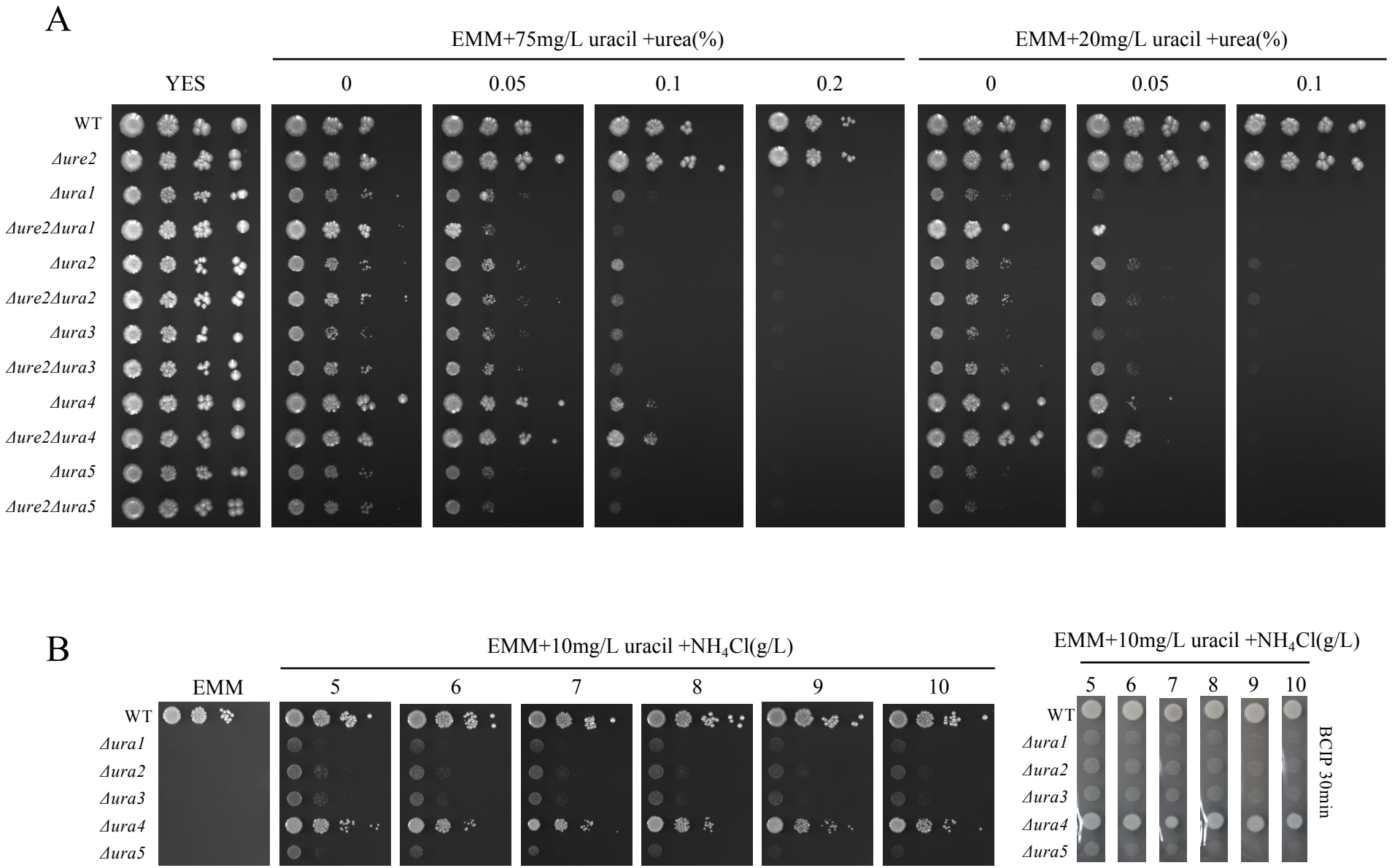

A
EMM+75mg/L uracil +urea(%)
EMM+20mg/L uracil +urea(%)
YES
0
0.05
0.1
0.2
0
0.05
0.1
WT
Δure2
Δura1
Δure2Δura1
Δura2
Δure2Δura2
Δura3
Δure2Δura3
Δura4
Δure2Δura4
Δura5
Δure2Δura5
B
EMM+10mg/L uracil +NH4Cl(g/L)
EMM
5
6
7
8
9
10
WT
Δura1
Δura2
Δura3
Δura4
Δura5
EMM+10mg/L uracil +NH4Cl(g/L)
5
6
7
8
9
10
WT
Δura1
Δura2
Δura3
Δura4
Δura5
BCIP 30min

Fig S4