


ORIGINAL ARTICLE

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A joint PCR-based gene-targeting method using electroporation in the pathogenic fungus *Trichosporon asahii*

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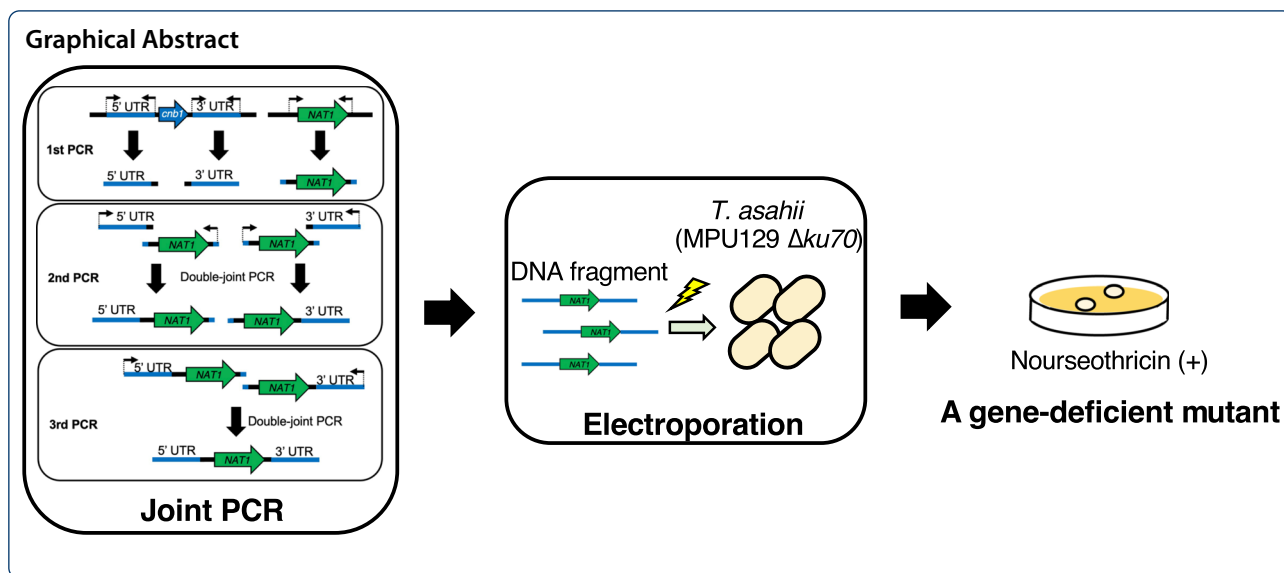
Abstract

Trichosporon asahii is a pathogenic fungus that causes deep-seated fungal infections in immunocompromised patients. Established methods for generating gene-deficient *T. asahii* mutants exist, but the frequency of obtaining transformants by electroporation remains low. In the present study, we optimized the conditions for gene transfer by electroporation using a *ku70* gene-deficient mutant with high recombination efficiency. Introducing a DNA fragment by electroporation into *T. asahii* cells on Sabouraud dextrose agar to generate a *cnb1* gene-deficient mutant and incubating for 1 day led to the growth of approximately 100 transformants. When the incubation period was extended to 2 days or 5 days, however, only 2 or no transformants, respectively, were grown. The highest number of transformants was grown by electroporation when a square wave at 1.8 kV (9 kV/cm) was applied for 5 ms. In addition, the number of transformants increased with an increase in the length of the homologous region, and transformants did not grow when the homologous region was less than 500 base pairs. A DNA fragment was produced for deletion of the *cnb1* gene by joint PCR, and the *cnb1* gene-deficient mutant was obtained by introducing the DNA fragment by electroporation. These results indicate that DNA fragments produced by joint PCR can be used to generate gene-deficient mutants of *T. asahii* through gene transfer by electroporation.

Keywords: *Trichosporon asahii*, Mutant, Electroporation, Joint PCR, Gene targeting

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Introduction

Trichosporon asahii is a basidiomycete yeast that is widely distributed in the environment and isolated from human blood, sputum, skin, feces, and urine (Sugita et al. 2001; Zhang et al. 2011; Colombo et al. 2011; Gouba et al. 2014; Cho et al. 2015). *T. asahii* is a pathogenic fungus that causes severe deep-seated fungal infections in neutropenic patients (Walsh et al. 1992, 1993; de Almeida Júnior and Hennequin 2016; Duarte-Oliveira et al. 2017). While the mortality rate of deep-seated mycosis caused by *Candida albicans* is approximately 40%, that caused by *T. asahii* is approximately 80% (Krcmery et al. 1999; Girmenia et al. 2005). *T. asahii* is resistant to echinocandin antifungals, and infectious diseases caused by *T. asahii* often occur in the patients treated with micafungin (Goodman et al. 2002; Kimura et al. 2018, 2022). Moreover, *T. asahii* strains resistant to antifungals such as amphotericin B and fluconazole have been isolated from patients (Toriumi et al. 2002; Iturrieta-González et al. 2014). Therefore, revealing the infection mechanisms and drug-resistant systems of *T. asahii* is crucial.

Phenotypic analyses using gene-deficient mutants are important for clarifying the molecular mechanisms of infection and drug resistance in *T. asahii*. We established a method for generating a gene-deficient mutant of *T. asahii* using an *Agrobacterium tumefaciens*-mediated gene transfer system (Matsumoto et al. 2021). The efficiency of genetic recombination in *T. asahii* was increased by deleting the *ku70* gene that encodes the Ku70 protein, a subunit of the non-homologous end-joining repair enzyme (Matsumoto et al. 2021). The *ku70* gene-deficient mutant

exhibited the same proliferative ability in vitro and the same virulence in a silkworm infection model as the wild strain of *T. asahii* (Matsumoto et al. 2021). Therefore, the *ku70* gene-deficient mutant of *T. asahii* is useful as a parental strain for generating gene-deficient mutants by homologous recombination.

The development of simple methods for generating gene-deficient mutants requires the optimization of gene transfer methods and improved construction of gene-targeting plasmids. For deletion of a target gene by homologous recombination, a DNA fragment containing the 5'-UTR and 3'-UTR of the target gene is necessary. The DNA fragment can be produced by joint PCR without cloning using *Escherichia coli* (Yu et al. 2004). A method for producing a gene-deficient mutant of *Cryptococcus neoformans*, a closely related species of *T. asahii*, was established using a DNA fragment produced by joint PCR (Lin et al. 2015). The DNA fragment used for deletion of the target gene is amplified by PCR and introduced into *T. asahii* by electroporation. When the *ku70* gene-deficient mutant of *T. asahii* is used as the parent strain, however, the number of transformants obtained by electroporation is less than 10 (Matsumoto et al. 2021).

In the present study, we optimized the electroporation conditions for gene transfer into *T. asahii*. A gene-deficient mutant was generated using a DNA fragment produced by joint PCR under optimized conditions. Our findings suggest that a gene-deficient mutant of *T. asahii* can be efficiently generated by electroporation without gene cloning using *E. coli*.

Materials and methods

Reagents

Nourseothricin and G418 were purchased from Jena Bioscience (Dortmund, Germany) and Enzo Life Science, Inc. (Farmingdale, NY, USA), respectively.

Culture of *T. asahii*

The *T. asahii* strain (MPU129 *ku70* gene-deficient mutant) used in this study was generated as previously reported (Matsumoto et al. 2021). The strain have been deposited in MPU culture collection. The *T. asahii* MPU129 *ku70* gene-deficient mutant was grown on Sabouraud dextrose agar (SDA) containing G418 (50 µg/ml) and incubated at 27 °C for 2 days.

Preparing competent *T. asahii* cells

Competent *T. asahii* cells were prepared according to a previous report with slight modification (Matsumoto et al. 2021). The *T. asahii* MPU129 *ku70* gene-deficient mutant was spread on SDA and incubated at 27 °C for 1, 2, or 5 days. *T. asahii* cells on the agar were suspended in physiologic saline solution (2 ml), and the suspension was transferred to a 1.5 ml tube. The fungal cells were collected by centrifugation at 8000 rpm for 3 min (TOMY-MX100, TOMY Digital Biology Co. Ltd, Tokyo, Japan) and suspended by adding 1 ml of ice-cold water and centrifuged at 8000 rpm for 3 min. This washing process was repeated four times. The washed cells were suspended by adding 1 ml of 1.2 M sorbitol solution and centrifuged at 8000 rpm for 3 min. The obtained fungal cells were suspended with 0.2 ml of 1.2 M sorbitol solution as competent cells.

Electroporation

Electroporation was performed according to a previous report with slight modification (Matsumoto et al. 2021). The PCR-amplified 5'-UTR (*cnb1*) -*NAT1*-3'-UTR (*cnb1*) fragment (180 ng/2 µl) was added to the competent *T. asahii* cells (40 µl) and placed on ice for 15 min. The suspension was added to a 0.2 cm gap cuvette (Bio-Rad Laboratories, Inc.) and electroporated (time constant protocol: 1.8 kV, 5 ms) using a Gene Pulser Xcell (Bio-Rad Laboratories, Inc.). The cells were suspended by adding 500 µl YPD containing 0.6 M sorbitol and incubated at 27 °C for 3 h. After incubation, the cells were collected by centrifugation at 10,000 rpm for 5 min, suspended in 100 µl of physiologic saline solution, and applied to SDA containing nourseothricin (300 µg/ml). The cells were incubated at 27 °C for 3 days, and the growing colonies were isolated as *cnb1* gene-deficient mutant candidates.

Genotyping PCR

Genotyping PCR was performed according to a previous report with slight modification (Matsumoto et al. 2021). The transformants were grown on SDA containing nourseothricin (300 µg/ml). Colony PCR was performed using Primers-2 for *cnb1* genotyping (Table 1). The genome of transformants selected by the colony PCR was extracted using a Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA). The mutation of the genome of the transformants was confirmed by PCR using the primers shown in Table 1.

Amplification of DNA fragments by joint PCR

Joint PCR was performed according to a previous report with slight modification (Lin et al. 2015). The genome of *T. asahii* MPU129 strain was used as a template to amplify the 5'-UTR (*cnb1*) and 3'-UTR (*cnb1*), and the previously generated 5'-UTR (*cnb1*)-*NAT1*-3'-UTR (*cnb1*) was used as a template to amplify the *NAT1* gene (1st PCR). Double-joint PCR was performed using the PCR products obtained by the 1st PCR to amplify 5'-UTR(*cnb1*)-*NAT1* and *NAT1*-3'-UTR(*cnb1*) (2nd PCR). The 5'-UTR (*cnb1*)-*NAT1*-3'-UTR (*cnb1*) was amplified by double-joint PCR using the PCR product obtained by 2nd PCR (3rd PCR).

Results

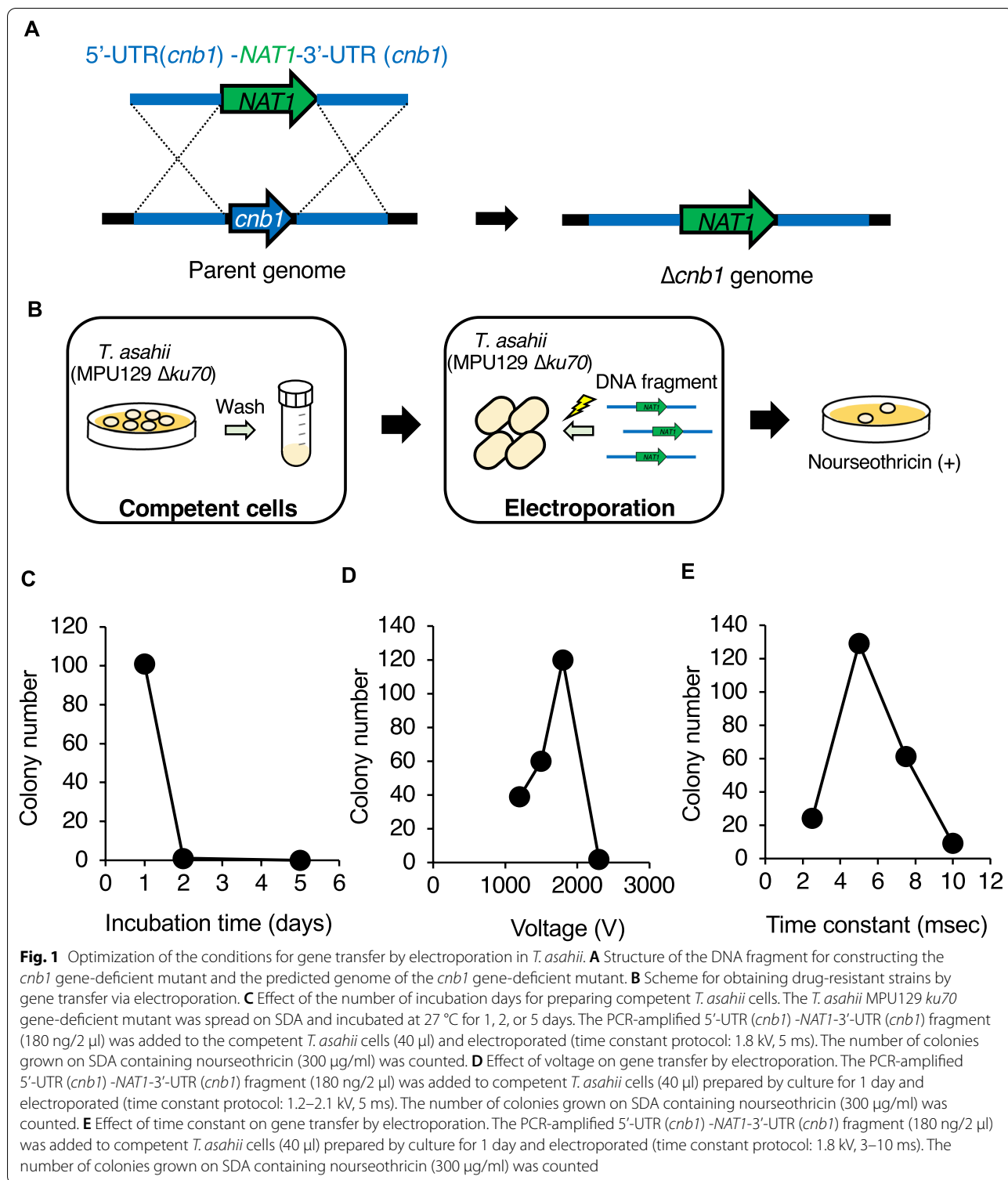
Optimization of electroporation conditions for the introduction of DNA fragments into *T. asahii*

In this study, we used a DNA fragment containing the 5'-UTR, the 3'-UTR of the *cnb1* gene, and the *NAT1* gene, a nourseothricin resistance gene (Fig. 1A). Increasing the gene transfer efficiency is necessary to optimize the conditions for the preparation of competent cells and electroporation (Fig. 1B). Proliferative phase cells often have higher competency than stationary phase cells and are used as competent cells for gene transfer in yeast (McGlincy et al. 2021). *T. asahii* cells were spread on SDA and incubated for 1, 2, or 5 days, respectively, and collected to prepare competent cells. When a DNA fragment was introduced by electroporation into a competent cell that had been cultured for 1 day to generate a *cnb1* gene-deficient mutant, approximately 100 colonies grew on SDA containing nourseothricin (Fig. 1C). On the other hand, only 2 colonies grew when the incubation period was 2 days, and no colonies were observed when the incubation period was 5 days (Fig. 1C). This finding suggests that the competency of *T. asahii* is decreased by longer incubation.

Next, we optimized the electroporation conditions. Approximately 120 colonies were grown on SDA containing nourseothricin at 1.8 kV (Fig. 1D). In the other

Table 1 Primers used in this study

Primers	Nucleic acid sequence
[Amplification of <i>cnb1</i> cassette]	
2000 bp	
<i>Fcnb1</i> (2000 bp)	CCGTGATCTGCTGCACGTTGCGGTCCG
<i>Rcnb1</i> (2000 bp)	CTGTTACCTCTGGCTACGACCCCTCCTC
1500 bp	
<i>Fcnb1</i> (1500 bp)	ACGAGCCTTGCGCTGGGCCTCCT
<i>Rcnb1</i> (1500 bp)	GCTCCTGCCGGTGGCTACGAGCAGCC
1000 bp	
<i>Fcnb1</i> (1000 bp)	CATTGCCGCCAACCTTGAGTGCTCGGAGC
<i>Rcnb1</i> (1000 bp)	GCCGCGCCCCCGCCAGCCGGGAAAC
500 bp	
<i>Fcnb1</i> (500 bp)	CGCGCCGCGCTGTCCATCGCTATTACAC
<i>Rcnb1</i> (500 bp)	CATACCCGCCCTTTCTATGTGACTGATACC
250 bp	
<i>Fcnb1</i> (250 bp)	CTGCGTTGGAGGACAGCTACTCGCCATGA
<i>Rcnb1</i> (250 bp)	ACATGTCATGATGTTTCATGGAAGGGGAAGAG
[Joint PCR]	
<i>cnb1</i> (5'UTR)	
<i>Fcnb1</i> (5'UTR)	ACGAGCCTTGCGCTGGGCCTCCT
<i>Rcnb1</i> (5'UTR)(<i>NAT1</i> inf)	AGCTCACATCCTCGCAGCGCAGTGCAATCTGGTCAGTCGGTCTGGCC
<i>cnb1</i> (3'UTR)	
<i>Fcnb1</i> (3'UTR)(<i>NAT1</i> inf)	TAGTTTCTACATCTTTCGCGCGCACACCGATGTGAGCGTAACGAG
<i>Rcnb1</i> (3'UTR)	GCTCCTGCCGGTGGCTACGAGCAGCC
<i>NAT1</i>	
<i>FNAT1</i> (5'UTR inf)	TGACCAAGATTGCACTGCGCTGCGAGGATGTGAGCTGGAGAGC
<i>RNAT1</i> (3'UTR inf)	ACATCCGTGTGTGCGCGGAAGAGATGTAGAACTAGCTTCTGTTTC
<i>cnb1</i> (5'UTR)- <i>NAT1</i>	
<i>Fcnb1</i> (5'UTR)	ACGAGCCTTGCGCTGGGCCTCCT
<i>RNAT1</i> (3'UTR inf)	ACATCCGTGTGTGCGCGGAAGAGATGTAGAACTAGCTTCTGTTTC
<i>NAT1</i> - <i>cnb1</i> (3'UTR)	
<i>FNAT1</i> (5'UTR inf)	TGACCAAGATTGCACTGCGCTGCGAGGATGTGAGCTGGAGAGC
<i>Rcnb1</i> (3'UTR)	GCTCCTGCCGGTGGCTACGAGCAGCC
<i>cnb1</i> (5'UTR)- <i>NAT1</i> - <i>cnb1</i> (3'UTR)	
<i>Fcnb1</i> (5'UTR)	ACGAGCCTTGCGCTGGGCCTCCT
<i>Rcnb1</i> (3'UTR)	GCTCCTGCCGGTGGCTACGAGCAGCC
[Genotyping]	
Primers-1 for <i>cnb1</i> genotyping	
F <i>cnb1</i> gene locus	GGAGTGAAGAAGGGCAGAGAGCAACAACAGCGGT
R <i>cnb1</i> gene locus	CCGTGATCGCATGGGGCGTGACAAAGTG
Primers-2 for <i>cnb1</i> genotyping	
F <i>cnb1</i> gene ORF	CGGCTCGGGTACGGTAGACTTCCAGGAGTTTGTCC
R <i>cnb1</i> gene ORF	AACAGGTCTCGAGCGTCATCTGTTGACGATGT



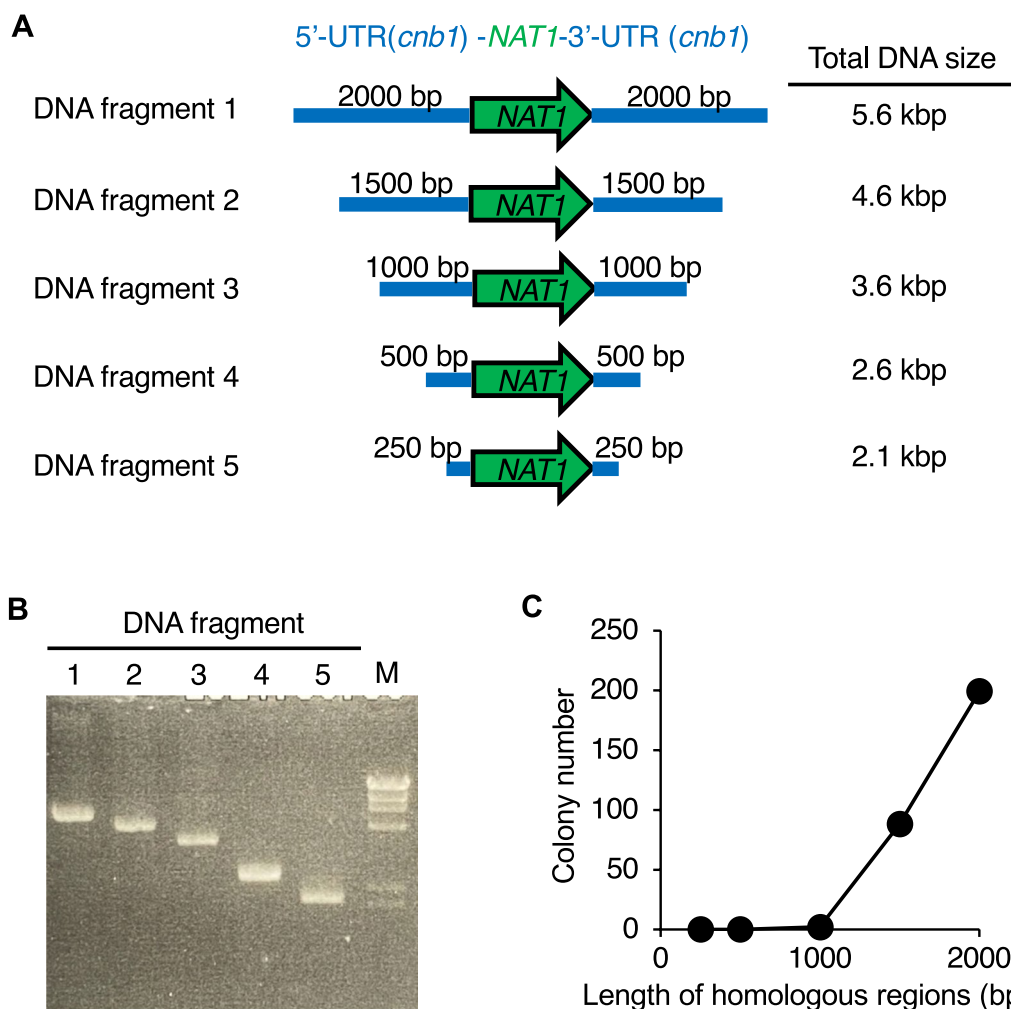
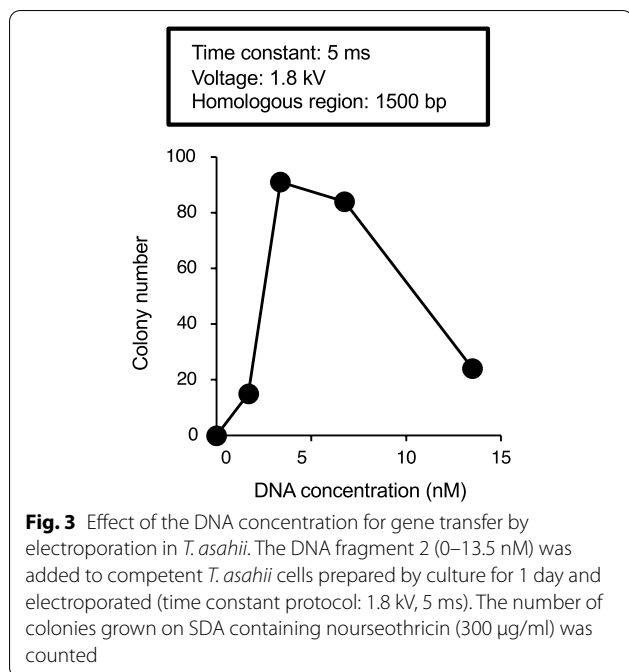


Fig. 2 Effect of the length of homologous regions for gene transfer by electroporation in *T. asahii*. **A** Illustration of DNA fragments used in this study. **B** Electrophoresis of DNA fragments amplified by PCR. **C** The PCR-amplified 5'-UTR (*cnb1*) -*NAT1*-3'-UTR (*cnb1*) fragments were added to the competent *T. asahii* cells prepared by culture for 1 day and electroporated (time constant protocol: 1.8 kV, 5 ms). The number of colonies grown on SDA containing nourseothricin (300 µg/ml) was counted

conditions, the number of colonies was lower than when the voltage was 1.8 kV (Fig. 1D). The highest number of colonies grew on SDA containing nourseothricin when the time constant was 5 ms (Fig. 1E). These results suggest that 1.8 kV (9 kV/cm) for 5 ms is the optimal condition for electroporation to introduce DNA fragments into the *T. asahii* MPU129 *ku70* gene-deficient mutant.

More than 500 base pairs of homologous regions are required for gene deletion by double crossover in the

filamentous fungi (Yu et al. 2004). On the other hand, DNA fragments, including longer homologous regions, are difficult to produce by joint PCR. We then examined the effect of the length of the homologous region on the efficiency of genetic recombination in *T. asahii*. DNA fragments with different lengths of homologous regions were amplified by PCR (Fig. 2A, B). The number of colonies grown on SDA containing nourseothricin increased as the length of the homologous region



increased (Fig. 2C). Colonies did not grow on SDA containing nourseothricin when the homologous region was less than 500 bp (Fig. 2C). The results suggest that a homologous region longer than 1500 bp enhanced the efficiency of gene transfer into the *T. asahii* cells.

Next, we examined the optimal concentration of DNA fragments for electroporation. When competent cells were electroporated with DNA fragment 2 having a homologous region of 1500 bp, more than 80 colonies grew at 3.4 and 6.8 nM (Fig. 3). In other conditions, the number of colonies was less than 30 (Fig. 3). These results suggest that a DNA fragment concentration of approximately 3.4–6.8 nM is optimal for gene transfer by electroporation.

Generation of a gene-deficient mutant using DNA fragments produced by joint PCR

Joint PCR is useful for obtaining DNA fragments without cloning in *E. coli* (Yu et al. 2004). We examined whether a *cnb1* gene-deficient mutant could be obtained using DNA fragments produced by joint PCR. Each joint PCR step amplified DNA fragments with

the predicted size, and a DNA fragment for generating the *cnb1* gene-deficient mutant was obtained (Fig. 4). Nourseothricin-resistant strains were obtained by introducing the DNA fragment into *T. asahii* by electroporation, which was optimized in this study. Among the 45 nourseothricin-resistant strains, 8 strains were deficient for the *cnb1* gene (Fig. 5, Table 2). These results suggest that the *cnb1* gene-deficient mutants of *T. asahii* were generated using DNA fragments amplified by joint PCR.

Discussion

Here, we developed a method to establish a *cnb1* gene-deficient mutant of *T. asahii* using PCR-amplified DNA fragments without gene cloning in *E. coli*. Using this method, it is theoretically possible to obtain a gene-deficient mutant of *T. asahii* within 1 week. Further experiments are needed to determine whether strains deficient in other genes can be obtained under these experimental conditions.

Optimizing the preparation of competent cells and electroporation conditions is very important for improving gene transfer (Edman and Kwon-Chung 1990; Wang 2018). The transformants in this study were obtained by introducing a drug-resistant gene via homologous recombination and non-homologous end-joining repair. In a previous study, we used competent cells cultured for 2–3 days, and the number of transformants obtained was less than 10 (Matsumoto et al. 2021). Competent cells incubated for 1 day had higher competency than those incubated for 2 days.

For generating gene-deficient mutants by electroporation, longer homologous regions should be used to increase the recombination efficiency (Ueno et al. 2007). On the other hand, the use of long DNA fragments decreased the gene transfer efficiency (Lamichhane et al. 2015). Moreover, long DNA fragments are difficult to produce by joint PCR (Bryksin and Matsumura 2010). Therefore, optimizing the length of the homologous regions is important toward increasing the efficiency of obtaining gene-deficient mutants. In *T. asahii*, a 1500-bp homologous region is needed to obtain gene-deficient mutant candidates by electroporation at a ratio of approximately 20%. The efficiency of homologous

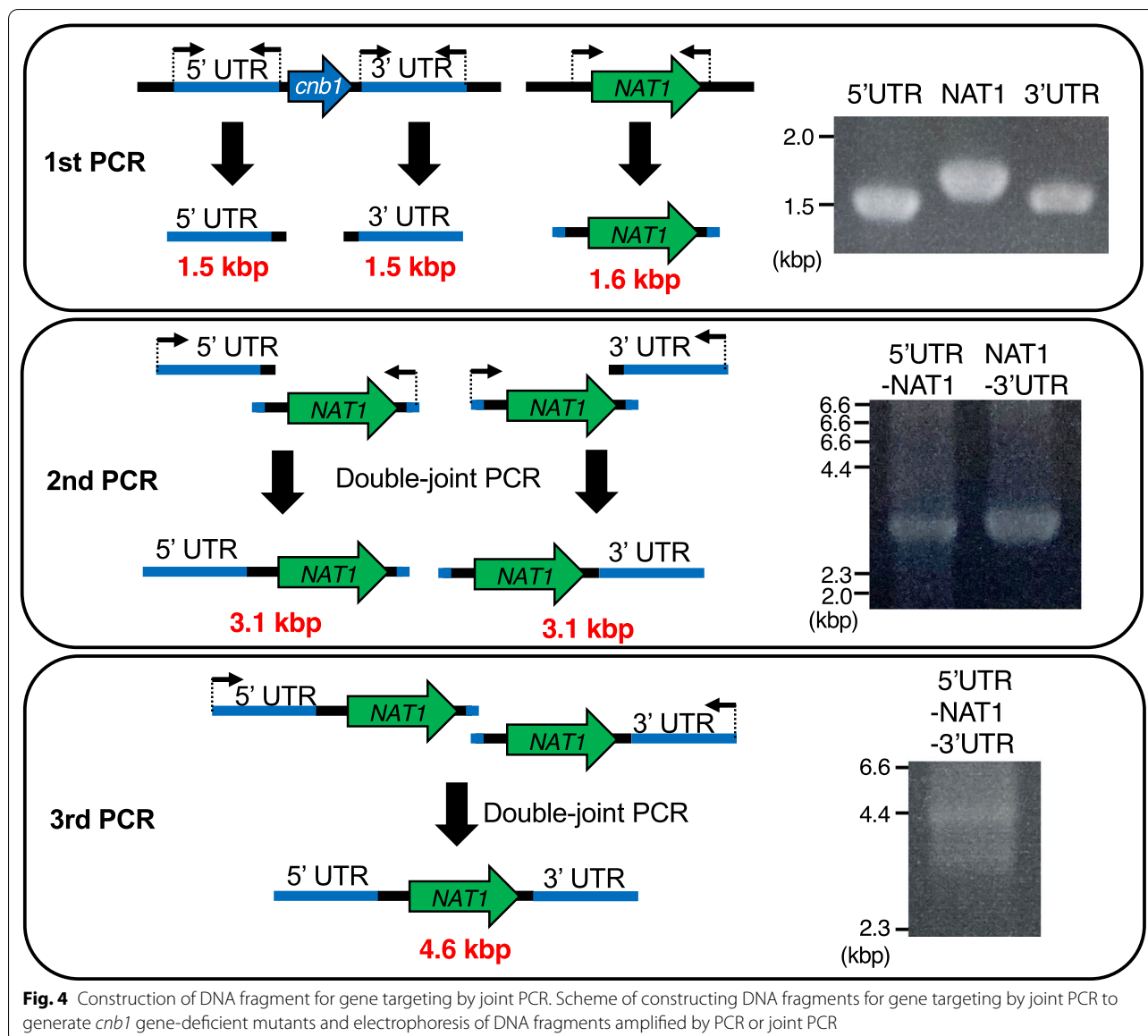


Fig. 4 Construction of DNA fragment for gene targeting by joint PCR. Scheme of constructing DNA fragments for gene targeting by joint PCR to generate *cnb1* gene-deficient mutants and electrophoresis of DNA fragments amplified by PCR or joint PCR

replacement in the *cnb1* gene region by joint PCR products in this study is similar to that in a previous study in which PCR products were amplified using a purified targeting plasmid (Matsumoto et al. 2021). Therefore, joint PCR products are useful for generating gene-deficient mutants in *T. asahii* by gene transfer via electroporation. A DNA fragment amplified by joint PCR for deletion of a target gene can be used to generate *cnb1* gene-deficient mutants in *T. asahii*. This method enabled us to produce DNA fragments for the deletion of target genes more

rapidly and conveniently than the conventional method of producing DNA fragments using *E. coli*. In a future study, we will generate gene-deficient mutants of *T. asahii* established in this study and analyze their pathogenicity functions.

In conclusion, we established a simple method for generating gene-deficient mutants in *T. asahii* via electroporation. The method might contribute to uncovering the molecular mechanisms of infection and drug resistance in *T. asahii*.

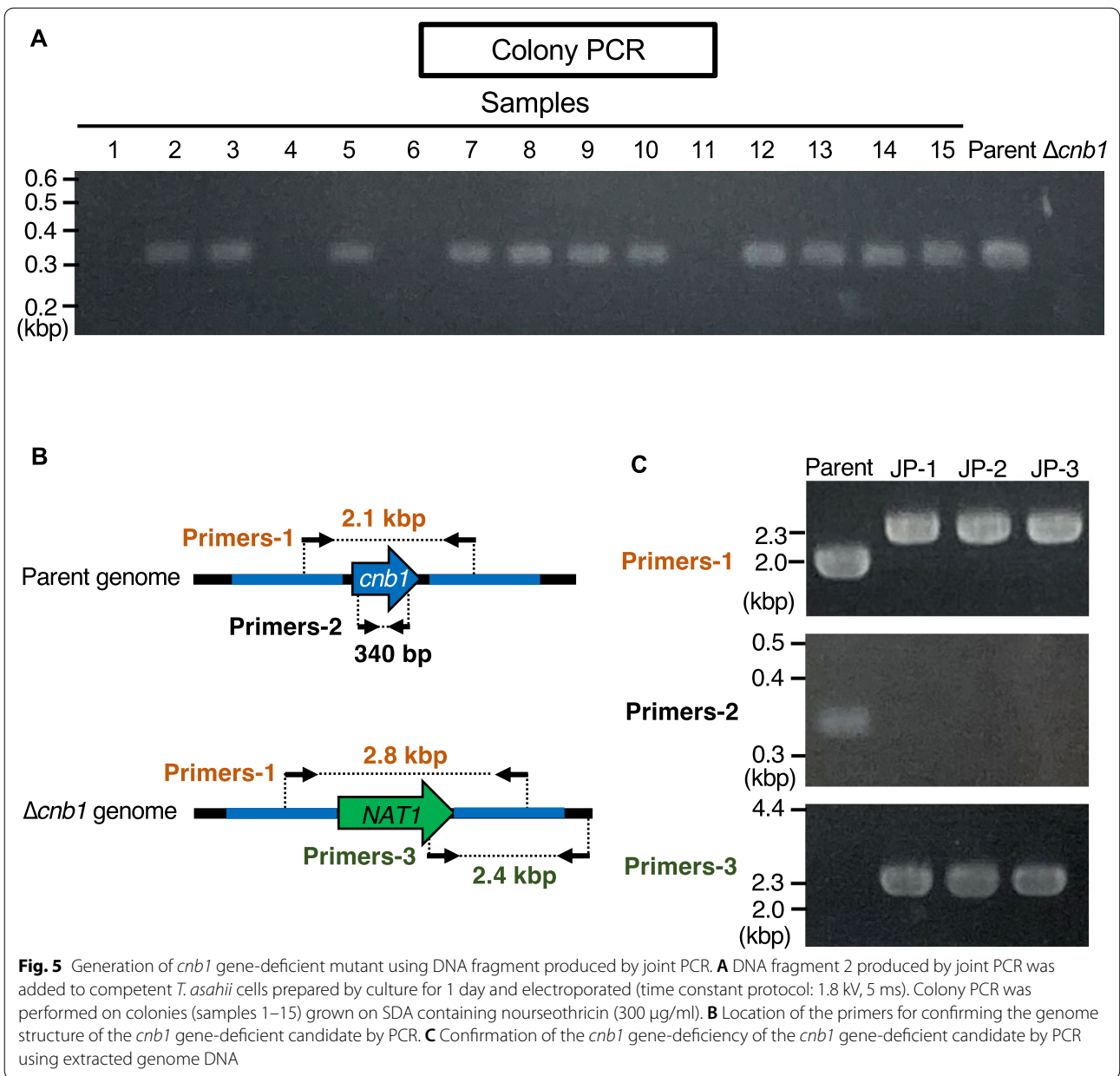


Table 2 Efficiency of homologous replacement in *cnb1* gene region

DNA fragment	Length of homologous regions (bp)	Total transformants	Homologous replacement ($\Delta cnb1$)	Efficiency (%) ($\Delta cnb1$ /total transformants)
Joint PCR product	1500	45	8	18%
DNA fragment 1 ^a	2000	21	4	19%

^a Data are cited from Matsumoto et al. (Matsumoto et al. 2021)

Acknowledgements

We thank Yu Sugiyama (Meiji Pharmaceutical University) for the technical assistances.

Author contributions

YM conceived and designed research. YM, AY, and TN conducted experiments. YT contributed to constructing the plasmid. YM, AY, and TN analyzed the data. YM wrote the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by JSPS KAKENHI Grant number JP20K07022 (Scientific Research (C) to Y.M.) and in part by the Research Program on Emerging and Re-emerging Infectious Diseases of the Japan Agency for Medical Research and Development, AMED (Grant number JP20fk0108135h0201 to T.S.).

Availability of data and materials

All data are presented in figures and tables within this article. Any material used in this study will be available for research purposes upon request.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare no competing interests.

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Received: 19 May 2022 Accepted: 2 July 2022

Published online: 14 July 2022

References

- Bryksin AV, Matsumura I (2010) Overlap extension PCR cloning: a simple and reliable way to create recombinant plasmids. *Biotechniques* 48:463–465. <https://doi.org/10.2144/000113418>
- Cho O, Matsukura M, Sugita T (2015) Molecular evidence that the opportunistic fungal pathogen *Trichosporon asahii* is part of the normal fungal microbiota of the human gut based on rRNA genotyping. *Int J Infect Dis* 39:87–88. <https://doi.org/10.1016/j.ijid.2015.09.009>
- Colombo AL, Padovan ACB, Chaves GM (2011) Current knowledge of *Trichosporon* spp. and Trichosporonosis. *Clin Microbiol Rev* 24:682–700. <https://doi.org/10.1128/CMR.00003-11>
- de Almeida Júnior JN, Hennequin C (2016) Invasive *Trichosporon* infection: a systematic review on a re-emerging fungal pathogen. *Front Microbiol* 7:1629. <https://doi.org/10.3389/fmicb.2016.01629>
- Duarte-Oliveira C, Rodrigues F, Gonçalves SM, Goldman GH, Carvalho A, Cunha C (2017) The cell biology of the *Trichosporon*-Host interaction. *Front Cell Infect Microbiol* 7:118. <https://doi.org/10.3389/fcimb.2017.00118>
- Edman JC, Kwon-Chung KJ (1990) Isolation of the URA5 gene from *Cryptococcus neoformans* var. *neoformans* and its use as a selective marker for transformation. *Mol Cell Biol* 10:4538–4544. <https://doi.org/10.1128/mcb.10.9.4538-4544.1990>
- Girmeria C, Pagano L, Martino B, D'Antonio D, Fanci R, Specchia G, Melillo L, Buelli M, Pizzarelli G, Venditti M, Martino P, Infection Program GIMEMA (2005) Invasive infections caused by *Trichosporon* species and *Geotrichum capitatum* in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. *J Clin Microbiol* 43:1818–1828. <https://doi.org/10.1128/JCM.43.4.1818-1828.2005>
- Goodman D, Pamer E, Jakubowski A, Morris C, Sepkowitz K (2002) Breakthrough trichosporonosis in a bone marrow transplant recipient receiving caspofungin acetate. *Clin Infect Dis* 35:E35–E36. <https://doi.org/10.1086/341305>
- Gouba N, Raoult D, Drancourt M (2014) Eukaryote culturomics of the gut reveals new species. *PLoS ONE* 9:e106994. <https://doi.org/10.1371/journal.pone.0106994>
- Iturrieta-González IA, Padovan ACB, Bizerra FC, Hahn RC, Colombo AL (2014) Multiple species of *Trichosporon* produce biofilms highly resistant to triazoles and amphotericin B. *PLoS ONE* 9:e109553. <https://doi.org/10.1371/journal.pone.0109553>
- Kimura M, Araoka H, Yamamoto H, Nakamura S, Nagi M, Yamagoe S, Miyazaki Y, Ogura S, Mitsuki T, Yuasa M, Kaji D, Kageyama K, Nishida A, Taya Y, Shimazu H, Ishiwata K, Takagi S, Yamamoto G, Asano-Mori Y, Uchida N, Wake A, Taniguchi S, Yoneyama A (2018) Micafungin breakthrough fungemia in patients with hematological disorders. *Antimicrob Agents Chemother* 62:324. <https://doi.org/10.1128/AAC.02183-17>
- Kimura M, Asano Mori Y, Sakoh T, Abe M, Ueno K, Hoshino Y, Nakamura S, Umeyama T, Yamagoe S, Miyazaki Y, Baba M, Okada C, Ogura S, Mitsuki T, Yamaguchi K, Yuasa M, Kaji D, Kageyama K, Nishida A, Taya Y, Ishiwata K, Takagi S, Yamamoto H, Yamamoto G, Uchida N, Wake A, Taniguchi S, Araoka H (2022) Factors associated with breakthrough fungemia caused by *Candida*, *Trichosporon*, or *Fusarium* species when the breakthrough fungemia developed in patients with hematological disorders. *Antimicrob Agents Chemother*. <https://doi.org/10.1128/AAC.02081-21>
- Krcmery V, Mateicka F, Kunová A, Spánik S, Gyarfás J, Sycová Z, Trupl J (1999) Hematogenous trichosporonosis in cancer patients: report of 12 cases including 5 during prophylaxis with itraconazole. *Support Care Cancer* 7:39–43
- Lamichhane TN, Raiker RS, Jay SM (2015) Exogenous DNA loading into extracellular vesicles via electroporation is size-dependent and enables limited gene delivery. *Mol Pharm* 12:3650–3657. <https://doi.org/10.1021/acs.molpharmaceut.5b00364>
- Lin X, Chacko N, Wang L, Pavuluri Y (2015) Generation of stable mutants and targeted gene deletion strains in *Cryptococcus neoformans* through electroporation. *Med Mycol* 53:225–234. <https://doi.org/10.1093/mmy/083>
- Matsumoto Y, Nagamachi T, Yoshikawa A, Yamazaki H, Yamasaki Y, Yamada T, Sugita T (2021) Development of an efficient gene-targeting system for elucidating infection mechanisms of the fungal pathogen *Trichosporon asahii*. *Sci Rep* 11:18270–18310. <https://doi.org/10.1038/s41598-021-97287-3>
- McGlincy NJ, Meacham ZA, Reynaud KK, Muller R, Baum R, Ingolia NT (2021) A genome-scale CRISPR interference guide library enables comprehensive phenotypic profiling in yeast. *BMC Genomics* 22:205–217. <https://doi.org/10.1186/s12864-021-07518-0>
- Sugita T, Ichikawa T, Matsukura M, Sueda M, Takashima M, Ikeda R, Nishikawa A, Shinoda T (2001) Genetic diversity and biochemical characteristics of *Trichosporon asahii* isolated from clinical specimens, houses of patients with summer-type-hypersensitivity pneumonitis, and environmental materials. *J Clin Microbiol* 39:2405–2411. <https://doi.org/10.1128/JCM.39.7.2405-2411.2001>
- Toriumi Y, Sugita T, Nakajima M, Matsushima T, Shinoda T (2002) Antifungal pharmacodynamic characteristics of amphotericin B against *Trichosporon asahii*, using time-kill methodology. *Microbiol Immunol* 46:89–93. <https://doi.org/10.1111/j.1348-0421.2002.tb02663.x>
- Ueno K, Uno J, Nakayama H, Sasamoto K, Mikami Y, Chibana H (2007) Development of a highly efficient gene targeting system induced by transient repression of YKU80 expression in *Candida glabrata*. *Eukaryot Cell* 6:1239–1247. <https://doi.org/10.1128/EC.00414-06>
- Walsh TJ, Lee JW, Melcher GP, Navarro E, Bacher J, Callender D, Reed KD, Wu T, Lopez-Berestein G, Pizzo PA (1992) Experimental *Trichosporon* infection in persistently granulocytopenic rabbits: implications for pathogenesis, diagnosis, and treatment of an emerging opportunistic mycosis. *J Infect Dis* 166:121–133. <https://doi.org/10.1093/infdis/166.1.121>
- Walsh TJ, Melcher GP, Lee JW, Pizzo PA (1993) Infections due to *Trichosporon* species: new concepts in mycology, pathogenesis, diagnosis and treatment. *Curr Top Med Mycol* 5:79–113

- Wang P (2018) Two distinct approaches for CRISPR-Cas9-mediated gene editing in *Cryptococcus neoformans* and related species. *Sphere*. <https://doi.org/10.1128/mSphereDirect.00208-18>
- Yu J-H, Hamari Z, Han K-H, Seo J-A, Reyes-Domínguez Y, Scazzocchio C (2004) Double-joint PCR: a PCR-based molecular tool for gene manipulations in filamentous fungi. *Fungal Genet Biol* 41:973–981. <https://doi.org/10.1016/j.fgb.2004.08.001>
- Zhang E, Sugita T, Tsuboi R, Yamazaki T, Makimura K (2011) The opportunistic yeast pathogen *Trichosporon asahii* colonizes the skin of healthy individuals: analysis of 380 healthy individuals by age and gender using a nested polymerase chain reaction assay. *Microbiol Immunol* 55:483–488. <https://doi.org/10.1111/j.1348-0421.2011.00341.x>

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