# Construction of the *Aspergillus nidulans pmtC*Δ*hogA*Δ*yA*Δ triple deletion strain by mating and benlate inducing

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**ARTICLE INFO**

**DOI:**10.46223/HCMCOUJS.tech.en.14.2.3208.2024

Received: January 24th, 2024
Revised: March 07th, 2024
Accepted: March 19th, 2024

*Keywords*:
Aspergillus nidulans; benlate; mating; triple deletion

**ABSTRACT**

In *Aspergillus nidulans*, construction of double or triple deletion strain is widely carried out to investigate genetic coordination, relation, or interaction. In this study, a fungal mutant, in which *pmtC*, *hogA*, and *yA* are deleted, is constructed for a recent experimental purpose. The mutation strain is screened and isolated by auxotrophic mutations from progenies of a sexual mating between the two *A. nidulans* fungi, *pmtC*Δ*yA*Δ deletion strain and *hogA*Δ deletion strain. The genotype of the strain obtained from the matting is confirmed by PCR indicating that the obtained strain contains deleted *hogA*, both wild-type *pmtC* and deleted *pmtC* deletion. Moreover, the obtained strain forms morphology with irregular edges, abnormal growth, and derived colony, which is like general morphology of diploid strains, suggesting that the mutant is a diploid strain. As benlate is a haploidization-inducer for the diploid strain, the spores of the diploid strain are spread on media with 0.05ppm of benlate. Forming colonies performing *pmtC*-like characteristics, such as poor development, impaired vegetation and limited conidiation, are considered as target haploid strains and confirmed by PCR. The PCR results show that, among four isolated strains, there is only one strain showing PCR band which is approximate to *pmtC* knock-out gene, indicating the strain carry *pmtC* deletion gene. As a result, the fungal strains, namely AnTLE36.HC, is a *pmtC*Δ*hogA*Δ*yA*Δ deletion strain.

## 1. Introduction

*Aspergillus nidulans* is a member of the filamentous fungi group, which plays an important role in medicine, agriculture, and industry. This species is often used as a model organism in the study of cell development and gene regulation. The life cycle of *A. nidulans* consists of separate reproductive (sexual) and vegetative (asexual) stages that characterize aspergilli, an important member of the filamentous fungal genus *Aspergillus.* In the sexual cycle, *A. nidulans* is homothallic, which means single ascospores give origin to self-fertile strains. On germination, the colorless septate hyphae are produced by a haploid vegetative spore (conidium).

Some of these are foot cells, it differentiates to form a multinucleate stalk (conidiophore) (Casselton & Zolan, 2002). The sexual cycle is concentrated in specialized organs, the fruiting bodies (cleistothecia or perithecia). Mature perithecia are present about 08 to 10 days after incubation of cultures at 37$^{o}$C. The mature cleistothecia is full of spheroidal shape asci, from 10 to 100,000 in cleistothecia of more than 100µm in diameter. There are eight brown-red ascospores, which are about 10µm in diameter, within each ascus. The mature ascus breaks very easily, liberating its eight ascospores, which germinate as quickly as the conidia (Pontecorvo, Roper, Chemmons, Macdonald, & Bufton, 1953). The fungicide methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl or benlate) is now the widely used haploidizing for *A. nidulans*. When cultured on a medium supplemented incorporating benlate causes growth retardation in diploid colonies, and long-term culture show marked instability, while the haploid bodies remain similar and relatively stable. Accordingly, the distinction between diploid and haploid strains can be made by culturing the strains on fungicide-containing media after 36h (Upshall, Giddings, & Mortimore, 1977).

*O*-mannosylation of proteins is an important process of *O*-glycosylation. In yeasts and filamentous fungi, *O*-mannosylation is required for the stabilization, classification, and localization of proteins, thereby influencing protein function and is integral to cell wall integrity, cell polarity and morphology of fungi (Lehle, Strahl, & Tanner, 2006). Protein *O*-mannosylation (Pmt) is initiated in the endoplasmic reticulum by transferring mannose from dolichol phosphate D-mannose to the serine and threonine of the secretory protein (Loibl & Strahl, 2013; Lommel & Strahl, 2009). This reaction is catalyzed by a group of protein *O*-mannosyltransferases (PMTs) (Strahl-Bolsinger, Gentzsch, & Tanner, 1999). There are three Pmt proteins in *A. nidulans*, consisting of PmtA, PmtB, and PmtC. Deletion of *pmtC* gene encoding protein PmtC leads to a decrease in fungal growth and development. However, the impaired growth of *pmtC*Δ deletant is partially restored under osmotic pressure (Le, Oki, Goto, & Shimizu, 2018).

To survive and thrive in the wild, the fungus must constantly adapt to the adverse environmental conditions it encounters. In *S. cerevisiae*, Hog1 is the final protein kinase in the HOG pathway, entering the nucleus to regulate transcription factors, controlling the activity of protein-coding genes in response to high osmotic conditions. Thus, knocking out the *hog1* gene would inactivate the HOG pathway, resulting in genes needed to respond to high osmotic environments being inactivated, limiting fungal growth under pressurized conditions high osmolarity (Hohmann, Krantz, & Nordlander, 2007). In *A. nidulans*, the *hogA* gene is a homolog of the *hog1* gene in *S. cerevisiae*, has been shown to be involved in response to high osmotic pressure (Han & Prade, 2002) Although *hogA* and *pmtC* function individually in hyperosmotic adaptation in *A. nidulans*, the genetic coordination of *hogA* and *pmtC* in fungal development under osmotic stress has not been characterized. In order to develop a study about the relationship between *hogA* and *pmtC* in *A. nidulans*, in this study, an *A. nidulans* mutant in which three genes *hogA*, *pmtC*, and *yA* are deleted, genotype and phenotype of the triple deletion strain were also analyzed. The construction of fungal deletants is partially carried out using the genetic transformation technique. However, the ability to survive and develop mutants, in which two or more genes are deleted, is greatly decreased. Therefore, to prevent this issue, in this study, the *A. nidulans* triple deletion strain was constructed by combining the mating and benlate inducing. *yA*, well characterized in *A. nidulans*, encodes a p-diphenol oxidase required for the synthesis of conidial green pigment. Deletion of the *yA* gene leads to the alteration of conidia color to yellow. As yellow conidia appear in *yA* deletant strains, potential triple deletants are preliminarily screened through the yellow conidia feature.

## 2. Material and methods

### *2.1. Strains, media, and growth conditions*

**Table 1**

*A. nidulans* strains used in this study

| Strains | Genotype | Required supplement | Source |
|---|---|---|---|
| TN02A7 | *nkuA*Δ, *pyroA4*, *pyrG89*, *riboB2* | Pyridoxine hydrochloride<br>Riboflavin<br>Uracil and uridine | (Nayak et al., 2006) |
| AnTLE8 | *nkuA*Δ, *pyroA4*, *pmtC*Δ::*AfpyrG*, *yA*Δ::*AfriboB* | Pyridoxine hydrochloride | (Le et al., 2018) |
| AnTLE36 | *nkuA*Δ, *hogA*Δ::*AfpyroA*, *pyrG89*, *riboB2* | Riboflavin<br>Uracil and uridine | This study |
| AnTLE8.HA | *nkuA*Δ, *hogA*Δ::*AfpyroA*, *pmtC*Δ::*AfpyrG*, *yA*Δ::*AfriboB* | Not required | This study |

*A. nidulans* strains used in this study are listed in Table 1. Growth conditions and media have been published previously (Le et al., 2018). All strains were grown on Aspergillus YGM medium (GMM medium with 0.5% yeast extract) with approximate supplement as a requirement of auxotrophic marker.

### *2.2. Mating method*

Spores were prepared from 10-day-old cultures and suspended in 0.1% Tween 80 $H_2O$. The concentrations of spore suspensions were adjusted to $1 \times 10^7$ spores $mL^{-1}$. The spore suspension (1µL x $10^4$ spores/mL) from the two parent strains was co-inoculated in YGM liquid media with the presence of uracil, uridine, riboflavin B, and pyridoxine HCl as supplements at 37$^o$C until hyphal matrix formed on the top of media. The hyphal matrix is next transferred to YGM or oatmeal media with no supplement, incubate at 25$^o$C for the formation of cleistothecia. Ascospores were collected from the cleistothecia, and then spread on YGM media with no supplement to pick up the targeted strain.

### *2.3. Isolation of haploid strains*

Benlate is used to induce haploidization of the diploid strain. The concentrations of benlate are 0.05ppm, 0.1ppm, 0.25ppm, 0.5ppm, 1ppm, and 1.5ppm, and the highest concentration of benlate that had no effect on fungal growth was chosen to screen haploid strains. Spore suspension of the diploid strain was prepared from 10-day-old cultures and suspended in 0.1% Tween 80 $H_2O$. About $10^2$ spores were spread on media with the presence of benlate, incubated at 25$^o$C for one day to pick up potential haploid strains. The potential haploid strains were inoculated in YGM media with no supplement to observe the growth morphology, and the genotype was confirmed by PCR with confirmed primers.

### *2.4. Genomic DNA extraction and PCR amplification*

Total genomic DNA (gDNA) extraction from fungal mycelia was carried out using UltraPure phenol-chloroform-isoamyl alcohol 25:24:1 (Invitrogen, ThermoFisher Scientific) according to the manufacturer's instruction. The gDNA was used as a template for PCR to

amplify targeted DNA fragments with specific primers. All oligonucleotide primers used in this study are listed in Table 2. The thermal conditions of PCR reactions were as follows: a holding stage at 95$^{o}$C for 10min; a cycling stage with 40 cycles, each cycle consisting of 15 seconds at 95$^{o}$C, 30 seconds at 55$^{o}$C, and 90 seconds at 72$^{o}$C; and a final extension at 72$^{o}$C for 05min. The PCR products are analyzed using agarose gel electrophoresis.

**Table 2**

Primers used in this study

| **Primer** | **Sequence (5' - 3')** |
|---|---|
| AnHogA.pyroA.UP | TCACTCTCGATCAAAGCATCCACATGATCGACAGC |
| AnHogA.pyroA.LP | CGATTCATTAGGGCTGCTGGATTACTAAGGTAATG |
| AnHogA.confirmF | CAGCCAGTTCTGCGATGTTTGTGAT |
| AnHogA.confirmR | AGGAGAATTCCTCAGTTGACCTAGC |
| AnPmtC.confirmF | GAAGACGACGATGACGATGACTACT |
| AnPmtC.confirmR | AGAACGAAGACGGCAGCCCCAACGT |

## 3. Results and discussion

In *A. nidulans*, the construction of double or triple-deletion strains is widely carried out in order to investigate genetic coordination, relation, and interaction. For the purposes of investigating the relationship between *pmtC* and *hogA*, a *pmtC*Δ*hogA*Δ double deletion strain has been generated by crossing *hogA*Δ with *pmtC*Δ. Next, cleistothecia are collected from the mating, and next, they disrupt their wall to harvest ascospores. Ascospores are spread on a selected medium for screening of *pmtC*Δ*hogA*Δ double deletion strain based on combinations of auxotrophic mutations in progenies. The two parental strains are AnTLE8, carrying *pmtC* deletion, *yA* deletion, and pyridoxine auxotrophic mutation, and AnTLE36, carrying uracil and riboflavin auxotrophic mutation, and *hogA* deletion (Table 1). Sexual production starts with the hyphal fusion of the two fungal parents, producing asci, in which the two haploid nuclei fuse, becoming a diploid nucleus. Next, meiosis happens in the diploid nucleus, consequently, eight haploid ascospores. The independent orientation of homologous chromosome pairs during meiosis results in the formation of the haploid ascospores that carry three deletion genes, *pmtC*, *yA*, and *hogA*, and are able to grow in media without supplement. Screening of ascospores on a medium with approximate supplements can pick up the expected deletion strain (Figure 1). The potential *pmtC*Δ*hogA*Δ*yA*Δ deletion strains are genetically confirmed by PCR according to deletion structures (Figure 2).

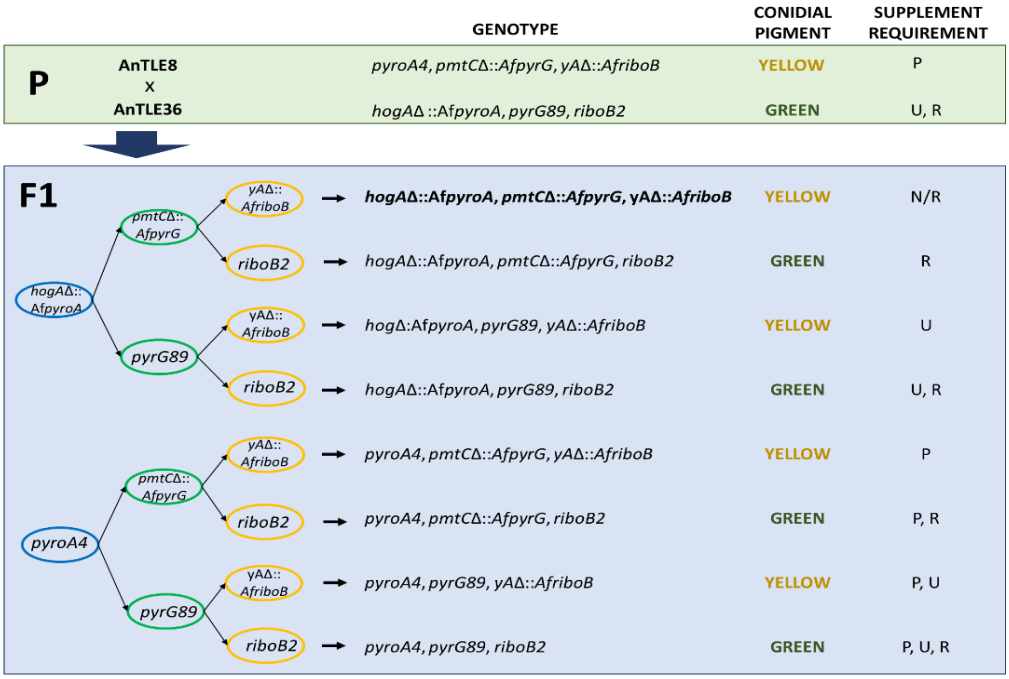


**Figure 1.** Mating of the two fungal strains, *pmtCΔyAΔ* deletant and *hogAΔ* deletant. Ascospores spores are screened according to auxotrophic mutation. The Ascospores spores are inoculated in YGM media without supplement to isolate expected strain. The genotype of the expected strain is bold. *Supplement requirement: P - Pyridoxine Hydrochloride, R - Riboflavin, U - Uracil and Uridine, N/R - Not required*

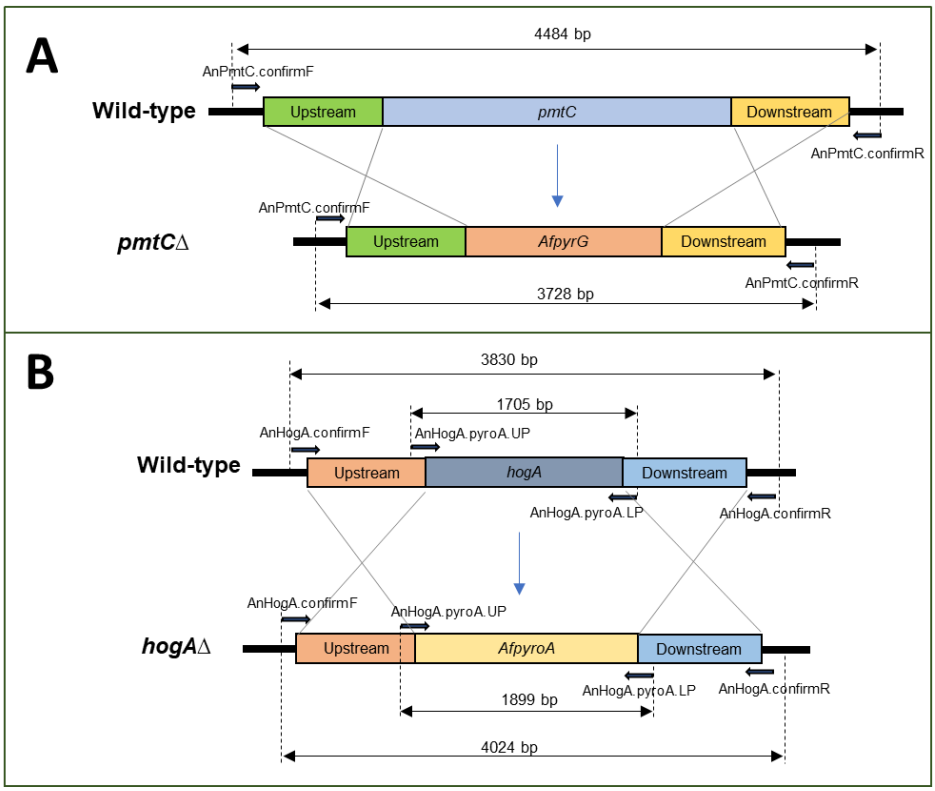


**Figure 2.** Structure of the *pmtC* deletion and *hogA* deletion genes. The *pmtC* and *hogA* genes are replaced by *AfpyrG* and *AfpyroA*, respectively. Gene deletion is confirmed by PCR to compare the size of amplification regions in the wild-type with those of the deletants.
(A) Structure of the *pmtC* deletion. (B) Structure of the *hogA* deletion

The strains, which grow on YGM without supplement and produce yellow conidia, are isolated for genotype confirmation by PCR. The PCR results by *hogA* confirm primers show that the two bands, amplified from both the wild-type and the obtained strain, are difficult to distingue due to a slight difference in the size of the two DNA fragments, 3,830bp from the wild-type and 4,024bp from the obtained strain (Figure 3A, land 4, 5, 6). Therefore, another PCR is carried out with other primer pairs, AnHogA.pyroA.UP/LP for analysis. Gel electrophoresis of PCR product amplified from the genome of the obtained strain by the primer pairs AnHogA.pyroA.UP/LP shows one band that is approximately to the *hogA*Δ gene, about 1,800bp, suggesting the strain carries *hogA* deletion in its genome (Figure 3A, land 7, 8, 9). On the other hand, there are two bands, about 4,400bp and 3,700bp, which are approximate to wild-type *pmtC* and deleted *pmtC*, respectively, presenting in the genome of the obtained strain, suggesting simultaneous presence of wild-type *pmtC* gene and deleted *pmtC* gene in the obtained strain (Figure 3A, land 1, 2, 3). The results are also recognized in other obtained strains (data not showed). By inoculating on a YGM medium, the obtained strain forms morphology with irregular edges, abnormal growth, and derived colony (Figure 3B), which is like the general morphology of diploid strains (Upshall et al., 1977). On the other hand, these strains produce yellow conidia and no appearance of either white or green conidia, suggesting the presence of *yA*Δ deletion in the strains. As a result, the *pmtC*Δ*hogA*Δ*yA*Δ deletion strain is unsuccessfully isolated from the mating between the two *pmtC*Δ*yA*Δ and *hogA*Δ strains, and the obtained strain is a diploid strain. PCR results and morphological characteristics indicate that the diploid strain has deleted *hogA* allele, deleted *yA* allele, and two different *pmtC* alleles, wild-type *pmtC*, and knock-out *pmtC*.

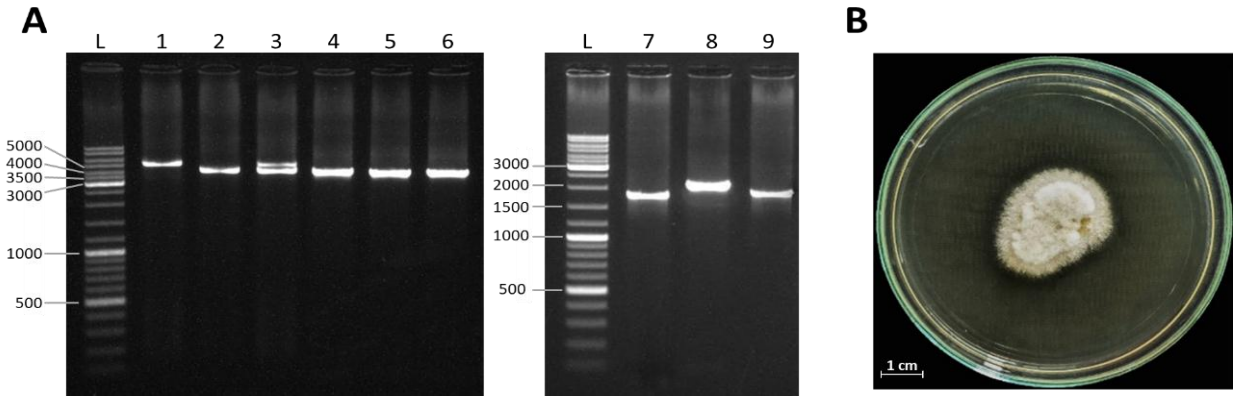


**Figure 3.** The diploid strain obtained from the mating between *pmtC*Δ*yA*Δ and *hogA*Δ deletants.
(A) Agarose gel electrophoresis of PCR products for genotype confirmation.
(B) Colonial formation of the diploid strain

L: GeneRuler DNA Ladder Mix;
1, 2, 3: PRC products amplified by the primer pairs AnPmtC.confirmF/R from the wild-type, *pmtC*Δ deletant, the expected strain from the mating between *pmtC*Δ and *hogA*Δ deletants, respectively;
4, 5, 6: PRC products amplified by the primer pairs AnHogA.confirmF/R from the wild-type, *hogA*Δ deletant, the expected strain from the mating between *pmtC*Δ and *hogA*Δ deletants, respectively;
7, 8, 9: PRC products amplified by the primer pairs AnHogA.pyroA.UP/LP from the wild-type, *hogA*Δ deletant, the expected strain from the mating between *pmtC*Δ and *hogA*Δ deletants

Previous studies reported that benlate functions as an inducer for haploidization in *A. nidulans* diploid strains. As benlate is a fungicide and exhibits fungal growth, the diploid strain is inoculated in various benlate concentrations to examine its survival ability. The 3-day culturing result shows that 1ppm of benlate is the maximum concentration in which the diploid strain develops. However, the fungus grows poorly and displays crinkled morphology with impaired

vegetative development. By decreasing the concentration of benlate to 0.05ppm, hyphal growth of the diploid strain is improved, resulting in normal colonial formation (Figure 4A). Therefore, the concentration of benlate used for haploidization is 0.05ppm. Spores of the diploid strain are collected, spread on media with 0.05ppm of benlate, and incubate at 37$^{o}$C (Figure 4B). After 3-day incubation, forming colonies are separately transferred into YGM medium plates to observe their growing morphology and used for PCR with the primer pairs AnPmtC.confirmF/R to examine their genotype. By continuously incubating for three days, colonies showing uniformity formation, and *pmtC*-like characteristics, such as poor development, impaired vegetation, and limited conidiation, are considered target haploid strains. The expected strains are confirmed by PCR with *pmtC* confirm primers. The PCR results show that, among four isolated strains, there is only one strain showing PCR band, which is approximately *pmtC* knock-out gene, 3,700bp, indicating the strain carries the *pmtC* deletion gene without wild-type *pmtC* gene (Figure 5). As a result, the fungal strains, namely AnTLE36.HC, is a *pmtC*Δ *hogA*Δ *yA*Δ deletion strain.

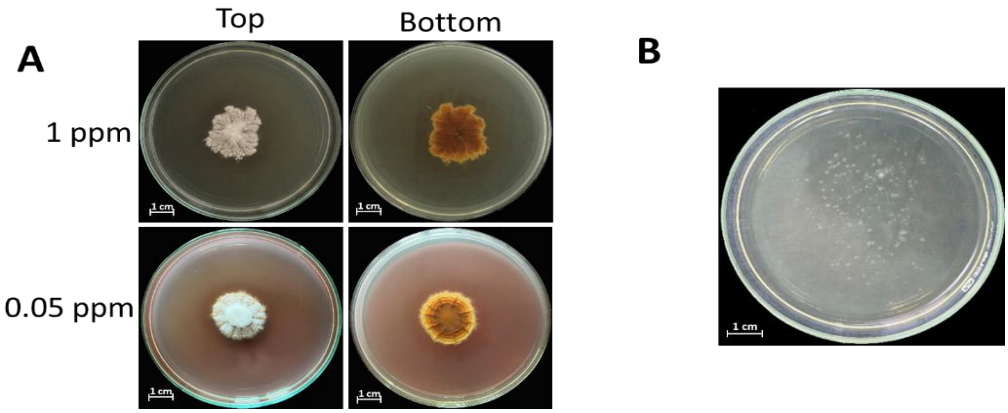


**Figure 4.** Growth of the diploid strain in the presence of benlate.
(A) Growth morphology of the diploid strain in 1ppm, and 0.05ppm benlate.
(B) Spore spreading of the diploid strain in 0.05ppm benlate

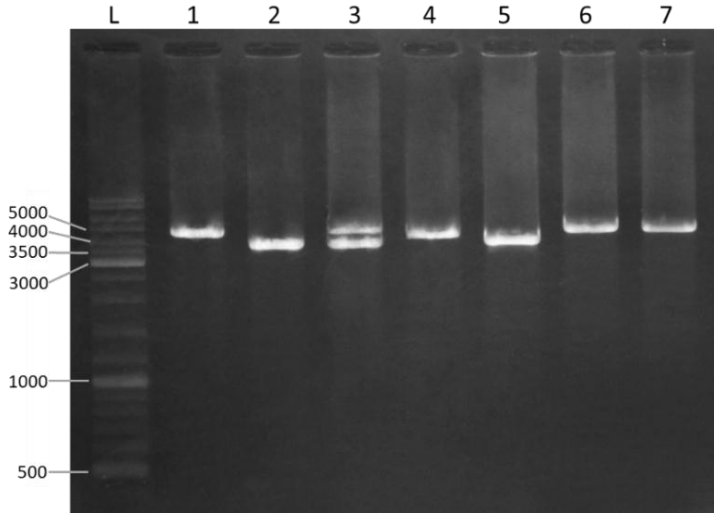


**Figure 5.** Agarose gel electrophoresis of PCR products by the primer pairs AnPmtC.confirmF/R for genotype confirmation of the diploid strain which is inoculated in 0.05ppm benlate for haploidisation

L: GeneRuler DNA Ladder Mix; 1: the wild-type; 2: *pmtC*Δ deletant; 3: the diploid strain; 4 - 7: colonies grown in media containing 0.05ppm of benlate

When inoculated on YGM media, the AnTLE36.HC displayed crinkled morphology, decreased vegetative production, and reduced conidia formation. Notably, colonial morphology of the AnTLE36.HC shows uniformity formation and yellow conidia, and no derivation of different colonial characteristics is detected (Figure 6). Moreover, the microscopic appearance of the AnTLE36.HC shows abnormal hyphal formations that are swollen and contain balloon structures. It supports the AnTLE36.HC is a haploid strain and carries three deleted genes, *pmtC*Δ *hogA*Δ, and *yA*Δ, in its genome.

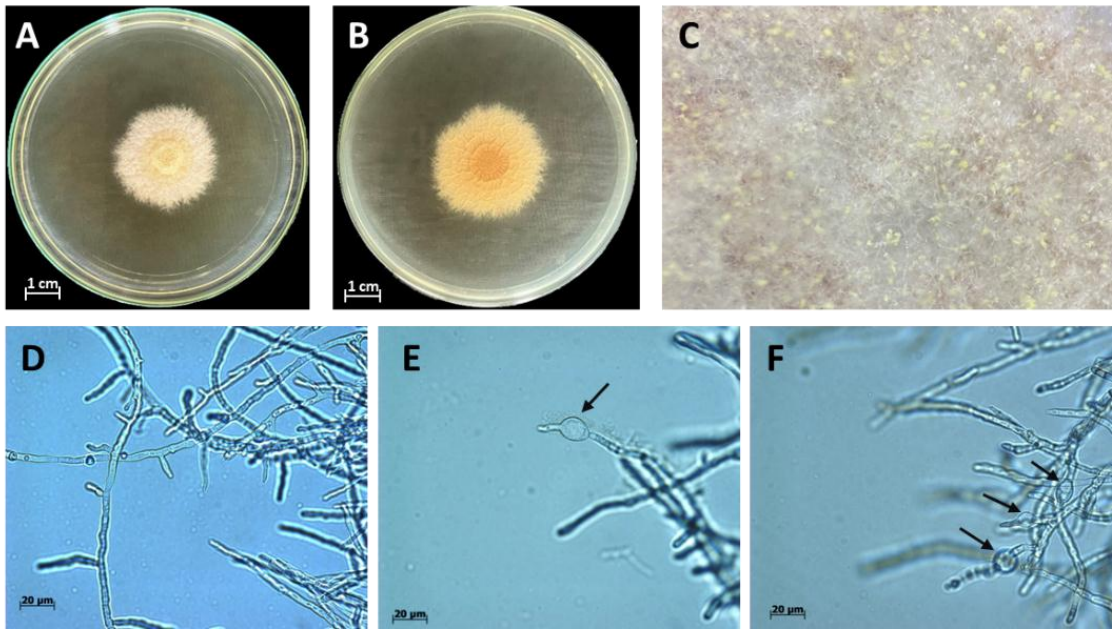


**Figure 6.** Growth morphology of the haploid strain that carries three deleted genes, *pmtC*Δ, *hogA*Δ, and *yA*Δ, in its genome. (A), (B), colonial morphology; (C), Close-up stereomicroscopic images of the strain (5x magnification); (D), (E), (F), Hyphal morphology. The arrows indicate balloon structures. Scale bar = 20µm.

*A. nidulans* is recognized as an excellent model organism for study in cell biology and gene regulation due to biological characteristics convenient for genetic manipulation. Impaired conidiation and vegetation in *the pmtC* deletion strain were restored under hyperosmotic pressure (Goto et al., 2009; Le et al., 2018). As *hogA* is responsible for fungal adaptation in hyperosmotic environment, a double deletant in which both *pmtC* and *hogA* genes were deleted to finger out an association between *pmtC*, *hogA*, and osmotic pressure in *A. nidulans*. Several experiments were done in order to construct a *pmtC*Δ*hogA*Δ deletant by transforming either *hogA* or *pmtC* deletion cassette into *pmtC*Δ deletant or *hogA*Δ deletant, respectively, but all experiment was unsuccessful. Therefore, the *pmtC*Δ*hogA*Δ deletant was harvested from the sexual mating between *hogA*Δ deletant and *pmtC*Δ deletant and isolated by auxotrophic mutation. Genotype of the two parent strains is showed in Table 1. Interestingly, all strains that are isolated from ascospore from the mating and media with no supplement added were diploid strains and carry two *pmtC* alleles, deleted *pmtC* and wild-type *pmtC* in their genome. No evidence was reported to demonstrate connection of *pmtC* with sexual development or cleistothecia formation in *A. nidulans*. In *Cryptococcus neoformans*, Pmt2, belonging to the PMT2 subfamily, was required for sexual development (Shimizu et al., 2014). Although inactivation of *hogA* does not have an influence on cleistothecia and ascospores formation of *A. nidulans*, the *hogA*Δ*steA*Δ double deletant produced no cleistothecia (Kawasaki, Sánchez, Shiozaki, & Aguirre, 2002). Moreover,

sexual mating between *hogA*Δ and *atfA*Δ strains was carried out in order to create a *hogA*Δ*atfA*Δ double deletion strain, but it was unsuccessful (Lara-Rojas, Sánchez, Kawasaki, & Aguirre, 2011). In *A. flavus*, the deletion of *sakA*, also known as *hogA*, affects the production of sclerotia, sexual reproductive structure (Tumukunde et al., 2019). On the other hand, *pmtC* has been previously reported as its essential role in fungal development. In *Beauveria bassiana*, deletion of *pmt2*, homologous of *pmtC*, eliminate fungal growth (Wang, Qiu, Chu, Ying, & Feng, 2014). In *Trichoderma reesei*, it is unable to obtain a *pmt2* mutant, but overexpression of *pmt2* resulte in increased conidial formation and abnormal polarity (Zhao et al., 2020). Similarly, *pmtC* also regulated developmental process, conidiogenesis, vegetative growth in other fungi, such as *Penicillium digitatum*, *Candida albicans*, *A. fumigatus* (Fang et al., 2010; Harries, Gandía, Carmona, & Marcos, 2015; Prill et al., 2005). Although the function of *pmtC* and *hogA* in haploidization has not been reported, the requirement of *pmtC* and *hogA* in fungal developmental processes, consisting of sexual reproduction, asexual reproduction, and vegetative growth, are well characterized. In our study, haploidization of ascospores from sexual cross of *hogA*Δ and *pmtC*Δ is abnormal, suggesting an association of *hogA* and *pmtC* on sexual reproduction in *A. nidulans*, which leaded to ineffective ascospore haploidization. It implies an unknown mechanism regulating haploidization through *pmtC*, or *hogA*, or both *pmtC* and *hogA* in *A. niudlans*. However, more studies should be done to obtain more obvious relations.

## 4. Conclusion

In conclusion, the *A. nidulans pmtC*Δ*hogA*Δ*yA*Δ triple deletion strain was successfully generated through benlate-induced ascospores from the mating of *pmtC*Δ*yA*Δ deletant and *hogA*Δ deletants. On the other hand, the deletion of *hogA* and *pmtC* appears to be endogenous factors that are associated with fungal sexual development in *A. nidulans*. Our study raises the question of how *hogA* and *pmtC* co-regulate fungal haploidization in partial and fungal sexual development in general, a question that should be addressed in future research.

## References

Casselton, L., & Zolan, M. (2002). The art and design of genetic screens: Filamentous fungi. *Nature Reviews Genetics*, *3*(9), 683-697. doi:10.1038/nrg889

Fang, W., Ding, W., Wang, B., Zhou, H., Ouyang, H., Ming, J., & Jin, C. (2010). Reduced expression of the *O*-mannosyltransferase 2 (AfPmt2) leads to deficient cell wall and abnormal polarity in *Aspergillus fumigatus*. *Glycobiology*, *20*(5), 542-552. doi:10.1093/glycob/cwp206

Goto, M., Harada, Y., Oka, T., Matsumoto, S., Takegawa, K., & Furukawa, K. (2009). Protein *O*-mannosyltransferases B and C support hyphal development and differentiation in *Aspergillus nidulans*. *Eukaryotic Cell*, *8*(10), 1465-1474. doi:10.1128/EC.00371-08

Han, K. H., & Prade, R. A. (2002). Osmotic stress-coupled maintenance of polar growth in *Aspergillus nidulans*. *Molecular Microbiology*, *43*(5), 1065-1078. doi:10.1046/j.1365-2958.2002.02774.x

Harries, E., Gandía, M., Carmona, L., & Marcos, J. F. (2015). The *Penicillium digitatum* protein O-mannosyltransferase Pmt2 is required for cell wall integrity, conidiogenesis, virulence and sensitivity to the antifungal peptide PAF26. *Molecular Plant Pathology*, *16*(7), 748-761. doi:10.1111/MPP.12232/SUPPINFO

Hohmann, S., Krantz, M., & Nordlander, B. (2007). Yeast osmoregulation. *Methods in Enzymology*, *428*(7), 29-45. doi:10.1016/S0076-6879(07)28002-4

Kawasaki, L., Sánchez, O., Shiozaki, K., & Aguirre, J. (2002). SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. *Molecular Microbiology*, *45*(4), 1153-1163. doi:10.1046/J.1365-2958.2002.03087.X

Lara-Rojas, F., Sánchez, O., Kawasaki, L., & Aguirre, J. (2011). *Aspergillus nidulans* transcription factor AtfA interacts with the MAPK SakA to regulate general stress responses, development and spore functions. *Molecular Microbiology*, *80*(2), 436-454. doi:10.1111/J.1365-2958.2011.07581.X

Le, T. T. H., Oki, A., Goto, M., & Shimizu, K. (2018). Protein O-mannosyltransferases are required for sterigmatocystin production and developmental processes in *Aspergillus nidulans*. *Current Genetics*, *64*(5), 1043-1056. doi:10.1007/s00294-018-0816-x

Lehle, L., Strahl, S., & Tanner, W. (2006). Protein glycosylation, conserved from yeast to man: A model organism helps elucidate congenital human diseases. In *Angewandte chemie - International edition* (Vol. 45, pp. 6802-6818). Weinheim, Germany: Wiley Press.

Loibl, M., & Strahl, S. (2013). Protein O-mannosylation: What we have learned from baker's yeast. In *Biochimica et Biophysica Acta - Molecular cell research* (Vol. 1833, pp. 2438-2446). Amsterdam, Netherlands: Elseiver Press.

Lommel, M., & Strahl, S. (2009). Protein O-mannosylation: Conserved from bacteria to humans. *Glycobiology*, *19*(8), 816-828. doi:10.1093/glycob/cwp066

Nayak, T., Szewczyk, E., Oakley, C. E., Osmani, A., Ukil, L., Murray, S. L., … Oakley, B. R. (2006). A versatile and efficient gene-targeting system for *Aspergillus nidulans*. *Genetics*, *172*(3), 1557-1566. doi:10.1534/genetics.105.052563

Pontecorvo, G., Roper, J. A., Chemmons, L. M., Macdonald, K. D., & Bufton, A. W. J. (1953). The genetics of *Aspergillus nidulans*. *Advances in Genetics*, *5*(C), 141-238. doi:10.1016/S0065-2660(08)60408-3

Prill, S. K. H., Klinkert, B., Timpel, C., Gale, C. A., Schröppel, K., & Ernst, J. F. (2005). PMT family of *Candida albicans*: Five protein mannosyltransferase isoforms affect growth, morphogenesis and antifungal resistance. *Molecular Microbiology*, *55*(2), 546-560. doi:10.1111/j.1365-2958.2004.04401.x

Shimizu, K., Imanishi, Y., Toh-e, A., Uno, J., Chibana, H., Hull, C. M., & Kawamoto, S. (2014). Functional characterization of PMT2, encoding a protein-O-mannosyltransferase, in the human pathogen *Cryptococcus neoformans*. *Fungal Genetics and Biology*, *69*(8), 13-22. doi:10.1016/j.fgb.2014.05.007

Strahl-Bolsinger, S., Gentzsch, M., & Tanner, W. (1999). Protein O-mannosylation. *Biochimica et Biophysica Acta - General Subjects*, *1426*(2), 297-307. doi:10.1016/S0304-4165(98)00131-7

Tumukunde, E., Li, D., Qin, L., Li, Y., Shen, J., Wang, S., & Yuan, J. (2019). Osmotic-adaptation response of sakA/hogA gene to aflatoxin biosynthesis, morphology development and pathogenicity in *Aspergillus flavus*. *Toxins*, *11*(1), Article 41. doi:10.3390/TOXINS11010041

Upshall, A., Giddings, B., & Mortimore, I. D. (1977). The use of benlate for distinguishing between haploid and diploid strains of *Aspergillus nidulans* and *Aspergillus terreus*. *Journal of General Microbiology*, *100*(2), 413-418. doi:10.1099/00221287-100-2-413

Wang, J. J., Qiu, L., Chu, Z. J., Ying, S. H., & Feng, M. G. (2014). The connection of protein O-mannosyltransferase family to the biocontrol potential of *Beauveria bassiana*, a fungal entomopathogen. *Glycobiology*, *24*(7), 638-648. doi:10.1093/GLYCOB/CWU028

Zhao, G., Xu, Y., Ouyang, H., Luo, Y., Sun, S., Wang, Z., … Jin, C. (2020). Protein O-mannosylation affects protein secretion, cell wall integrity and morphogenesis in *Trichoderma reesei*. *Fungal Genetics and Biology*, *144*(11), Article 103440. doi:10.1016/J.FGB.2020.103440