농학석사 학위논문

Cellular compartmentalization of zearalenone biosynthesis

지랄레논 생합성 효소의 세포 구획화 연구

2021 년 8 월

서울대학교 대학원

농생명공학과 식물미생물학 전공

전민호

Cellular compartmentalization of zearalenone biosynthesis

A dissertion submitted in partial
fulfillment of the requirement for
the degree of

Master Degree

to the Faculty of
Department of Agricultural Biotechnology

at

SEOUL NATIONAL UNIVERSITY

By

Minho Jun

August, 2021

농학석사 학위논문

Zearalenone 생합성 효소의 세포 구획화 연구

지도교수 손 호 경

이 논문을 농학석사 학위논문으로 제출함
2021년 8월

서울대학교 대학원
농생명공학부 식물미생물전공
전 민 호

전민호의 농학석사 학위논문을 인준함
2021년 8월

위 원 장 _____

부 위 원 장 _____

위 원 _____

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Cellular Compartmentalization of Zearalenone biosynthesis

UNDER THE DIRECTION OF
DR. HO-KYOUNG SON

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

BY MINHO JUN

MAJOR IN PLANT MICROBIOLOGY
DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

AUGUTST 2021

APPROVED AS A QUALIFIED THESIS OF MINHO JUN
FOR THE DEGREE OF MASTER OF SCIENCE
BY THE COMMITTEE MEMBERS

CHAIRMAN

VICE CHAIRMAN

MEMBER

Abstract

Cellular compartmentalization of Zearalenone biosynthesis

Minho Jun

Major in Plant Microbiology

Department of Agricultural Biotechnology

The Graduate School

Seoul National University

Fusarium graminearum is a plant pathogen that causes Fusarium head blight (FHB) in major cereal crops such as wheat, barley, corn, and rice worldwide. Not only does this fungus causes destructive yield losses, it also accumulates mycotoxins, zearalenone (ZEA) and trichothecene (DON and NIV), on infected grains, which pose serious threat to human and animal health. The structure of ZEA is similar to estrogen, and it binds to estrogen receptors of mammals and interferes with effect of estrogen, resulting in reproductive dysfunction. Previous studies characterized ZEA biosynthetic genes (*PKS4*, *PKS13*, *ZEB1*, and *ZEB2*) and their mechanism of action in ZEA biosynthesis of *F. graminearum*. However, we still know relatively little about subcellular localization of the ZEA biosynthetic enzymes and the cellular machinery required for ZEA production. In this study, green

fluorescent protein (GFP) was tagged to Pks4, Pks13, and Zeb1, and all proteins were revealed to localize in specific organelles under ZEA inducing condition. Moreover, Zeb1-Red fluorescent protein (RFP) fusion protein was expressed in Pks4-GFP and Pks13-GFP background strains. The results showed that Pks4, Pks13, and Zeb1 were co-localized in specific cellular organelles. FM4-64 staining and expression of RFP tagged with SKL, which is peroxisome targeting signal (PTS), were performed to reveal the specific organelles. I found that all of ZEA biosynthetic enzymes are exclusively localized in peroxisomes, suggesting that zearalenone is expected to be biosynthesized in peroxisome. This study will provide information on where the polyketide secondary metabolites are biosynthesized, and let us develop a novel mycotoxin reduction strategy.

Keywords : *Fusarium graminearum*, Zearalenone, Secondary metabolite, Cellular compartmentalization, Peroxisome
Student Number : 2019-26417

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The filamentous ascomycete fungus *Fusarium graminearum* causes Fusarium head blight (FHB) in wheat, barley and rice (Leslie and Summerell, 2006), which causes serious yield losses worldwide (Windels, 2000; Goswami and Kistler, 2004). In addition, this fungus contaminates grains by accumulating mycotoxins, such as trichothecenes and zearalenone (ZEA), which are toxic to humans and to livestock (Desjardins and Proctor, 2007). Zearalenone (6-[10-hydroxy-6-oxo-trans-1-undecenyl]-β-resorcyclic acid lactone) is a non-steroidal, polyketide mycotoxin produced by *F. culmorum, F. equiseti, F. crookwellense* and mainly by *F. graminearum* (Marasas *et al.*, 1984; Desjardins, 2006; Zinedine *et al.*, 2007). ZEA induces hyperestrogenic effects in mammals, resulting in reproductive dysfunctions in livestock, especially in swine and laboratory animals (Desjardins, 2006).

ZEA biosynthetic genes are located in the gene cluster which is typical of fungal secondary metabolite biosynthesis (Steele *et al.*, 1974; Mirocha *et al.*, 1978). The gene cluster consists ZEA biosynthetic genes *PKS4*, *PKS13*, *ZEB1*, and *ZEB2*. Each of gene encodes reducing polyketide synthase (PKS), nonreducing PKS, isoamyl alcohol oxidase, and basic leucine zipper (bZIP) transcription factor respectively (Kim *et al.*, 2005; Gaffoor and Trail, 2006;

Lysøe *et al.*, 2006). The ZEA biosynthetic pathway is initiated by Pks4, which induces the condensation of carbons from one acetyl-CoA and five malonyl-CoA molecules, resulting in structuring an alkene. After this alkene is delivered to Pks13, the PKS synthase continues to extend the ZEA chain by adding three additional malonyl-CoA molecules. Then, the unreduced ketones undergo intramolecular aromatic reactions, resulting in formation of an aromatic ring and a macrolide ring structure containing a lactone bond (Gaffoor and Trail, 2006), called β-zearalenol. Zeb1 catalyzes the conversion of β-zearalenol to ZEA by oxidation of hydroxyl group (Kim et al., 2005). Zeb2 acts as a potential transcriptional activator of genes that regulate ZEA biosynthesis.

A mechanism of exocytosis for secondary metabolite has recently been proposed in *Aspergillus parasiticus* (Chanda *et al.*, 2009). Toxisomes, typical cellular structures containing toxin biosynthesis enzymes, are usually found with other secondary metabolite; mycotoxin, such as aflatoxin, trichothecenes, and melanin. Aflatoxin is synthesized through endosome-derived toxisome (Kenne *et al.*, 2018). Trichothecenes are biosynthesized through golgi-derived toxisome (Kistler and Broz, 2015). Melanin is produced in endosome-derived vesicle, transported to cell wall in later step (Upadhyay *et al.*, 2016). Recent study proposed toxisomes may sequester trichothecene and melanin products and intermediates, thus protecting the

cell from their toxic activity and promoting pathway efficiency (Kenne *et al.*, 2014).

In this study, I tagged green fluorescent protein (GFP) on ZEA biosynthetic enzymes and further investigated their subcellular localization for actual functioning. The objectives of this study were to investigate the cellular compartmentalization of ZEA biosynthetic enzymes, and to identify the organelles in which enzymes work.

MATERIALS AND METHODS

I. Fungal strains and culture condition

The *F. graminearum* wild-type strain Z-3639 was used for generating transgenic strains (Bowden and Leslie, 1999). The wild-type and all transgenic strains were stored as mycelia and conidia in a 20% glycerol solution at -70 °C (Table 1). The culture media were prepared and used following the *Fusarium* laboratory manual (Leslie and Summerell, 2006).

II. DNA extraction, Southern blotting and PCR

Genomic DNA was isolated from freeze-dried mycelium powder (Leslie and Summerell, 2006). Standard techniques were used for restriction endonuclease digestion, agarose gel electrophoresis and Southern blot analysis (Russell and Sambrook, 2001). The primers used in this study were synthesized by an oligonucleotide synthesis facility (Bionics, Seoul Korea, Table 2). PCR procedures were performed as the manufacturer's instructions (Takara Bio Inc., Otsu, Japan).

Table 1. *F. graminearum* strains used in this study.

Strain	Genotype	Source or reference
Z-3639	Wild-type	Bowden and Leslie, 1999
Pks4-GFP	*PKS4::GFP-GEN*	This study
Pks13-GFP	*PKS13::GFP-GEN*	This study
Zeb1-GFP	*ZEB1::GFP-HYG*	This study
Pks4-GFP::Zeb1-RFP	*PKS4::GFP-GEN; ZEB1::RFP-HYG*	This study
Pks13-GFP::Zeb1-RFP	*PKS13::GFP-GEN; ZEB1::RFP-HYG*	This study
Pks4-GFP::SKL-RFP	*PKS13::GFP-GEN; SKL::RFP-HYG*	This study
Pks13-GFP::SKL-RFP	*PKS13::GFP-GEN; SKL::RFP-HYG*	This study

Table 2. Primers used in this study.

Primer	Sequence (5' → 3')	Description
Zeb1-5F	CGCAACACTGGCCATGACTATCT	Forward and reverse primers for amplification
Zeb1-5R	atcacaaacatgcgcgtagaggtcTAAAGCTGACTTGCACATTCTTCC	of 5' flanking region of *FGSG_15982* with tail for geneticin resistance gene cassette fusion
Zeb1-3F	ggcggaggcggaggcTTCTAGTTTCTAGCTCTGTGTTCT	Forward and reverse primers for amplification
Zeb1-3R	CAACTTGTATTGCGTGATGGTGGA	of 3' flanking region of *FGSG_15982* with tail for geneticin resistance gene cassette fusion
Zeb1-5N	CCTTGCGAGGAGGCTTCACC	Forward and reverse nest primers for third
Zeb1-3N	CCTCGACGGTGCGGGATTA	fusion PCR for amplification of *FGSG_15982* GFP tagging construct
PKS4/5F	TTTACTTTGTTGACGACGAGCGAGTA	Forward and reverse primers for amplification
PKS4/5R	gcctccgcctccgccAGATACCGTAACCAACTTGCTAGTAC	of 5' flanking region of *FGSG_17745* with tail

		for geneticin resistance gene cassette fusion
PKS4/3F	gacctctacgcgcatgtttgtgatTGTGCAACTAGCGAGCATAACACTTC	Forward and reverse primers for amplification
PKS4/3R	GAACGATGTGTTTGTGATGATGTGAA	of 3' flanking region of *FGSG_17745* with tail
		for geneticin resistance gene cassette fusion
PKS4/5N	AAACGCAAGCTTCTCACGGACAC	Forward and reverse nest primers for third
PKS4/3N	GCTTCCTCCGAGTTTGTATCAGTGG	fusion PCR for amplification of *FGSG_17745*
		GFP tagging construct
PKS13/5F	CCTTGATTGACGGCCATAGGTTG	Forward and reverse primers for amplification
PKS13/5R	gcctccgcctccgccCCCCGCCTCGTTAAAGAACTCA	of 5' flanking region of *FGSG_15980* with tail
		for geneticin resistance gene cassette fusion
PKS13/3F	gacctctacgcgcatgtttgtgatAGGCTATTTCATAGGCAGTTTAGTTTGG	Forward and reverse primers for amplification
PKS13/3R	GGTCGCATTCTACCAAACCTACTCAT	of 3' flanking region of *FGSG_15980* with tail
		for geneticin resistance gene cassette fusion

PKS13/5N	CCTGTGGGCACAAAGTTCGTAGC	Forward and reverse nest primers for third fusion PCR for amplification of *FGSG_15980* GFP tagging construct
PKS13/3N	GACTATCCCCCATCCAAATAACTTGTTC	

III. Subcellular localization

To investigate localization of ZEA biosynthetic enzymes and the endosomal compartments of fungal strains, N-(3-triethylammoniumpropyl)-4-(p-diethylaminophenyl-hexatrienyl) pyridinium dibromide (FM4-64; Invitrogen, Carlsbad, CA, USA) and 7-Amino-4-chloromethylcoumarin (CMAC) were used in this study (Fischer-Parton *et al.*, 2000; Shoji *et al.*, 2006). To observe peroxisomal localization of ZEA biosynthetic enzymes, SKL-RFP insertion mutants (*HYG-RFP-SKL*) were constructed (Min *et al.*, 2012).

Mycelia grown in starch-glutamate (SG) or potato dextrose broth (PDB) media were used for cytological observations. FM4-64 staining was performed as follows. Harvested mycelia were washed twice with 1X phosphate buffered saline (PBS) buffer and re-submerged with fresh medium containing 10 uM FM4-64 for 20 min at room temperature (RT) in dark condition. After washing again by 1X PBS buffer twice, the cultures were fixed by 2% paraformaldehyde (PFA) for 30 min at RT (Fischer-Parton et al., 2000).

For CMAC staining, sample was washed by 1X PBS buffer twice and fixation was performed with 2% PFA for 30 min at RT before staining. The cultures were submerged with 10 uM CMAC staining medium, and incubated for 40 min at RT in dark condition. After washing with 1X PBS buffer twice, the sample was re-suspended with dye-free medium and

additionally incubated for 30 minutes at 30°C in dark condition for de-staining (Shoji et al., 2006).

IV. Toxin analysis

To induce ZEA production, SG and PDB media were used for culturing fungal strains (Park *et al.*, 2016). SG media were filtered by miracloth, collected at 15mL conical tube. Collected culture was diluted by ethyl acetated as 1:4 ratio at 8mL glass vial. The glass vial was vigorously shaked (200 rpm) for 30 min, and waited 5 min for layer separation. The upper layer was transferred to 2 mL glass vial and dried by speed-vac for 30 min. The residue was dissolved in the mobile phase (70% of methanol solution) and analyzed with a Shimadzu LC-6A HPLC equipped with an RF-10A XL fluorescence detector (Shimadzu) (Lee *et al.*, 2011).

V. Microscopic observations

Leica DM4B microscope with fluorescent filters L5 (order no. 11504166) and Y3 (order no. 11504169) equipped with a Leica DMC6200 camera (Leica Microsystems, Germany) was used for obtaining differential interference contrast (DIC) and fluorescence images.

RESULTS

Localization of ZEA biosynthetic mutants

In order to observe subcellular locations of Zeb1-GFP, Pks4-GFP, and Pks13-GFP better, Zeb2OE (Park et al., 2015), a strain that overexpresses the ZEA biosynthetic genes, was prepared and observed, yet green fluorescence was observed throughout hyphal cells (Fig. 1). However, lots of observations have confirmed that some of the hyphal cells are presenting the fluorescence at some organelles (Fig. 2).

Instead of using *ZEB2* overexpression strains, the wild type strain expressing Zeb1-GFP, Pks4-GFP, or Pks13-GFP were used for localization analysis. We could observe that all of ZEA biosynthetic enzymes fused with GFP were exclusively localized in some parts of hyphal cells (Fig. 3).

Since it was observed that the ZEA-synthesizing proteins were located in some part of mycelia, double tagging mutants in which red fluorescence protein (RFP) was tagged at Zeb1 with GFP tagging at Pks4 or Pks13 mutants were constructed and observed to check whether the proteins are located at the same location at the same time. I found that both GFP and RFP fluorescent signals were overlapped (Fig. 4).

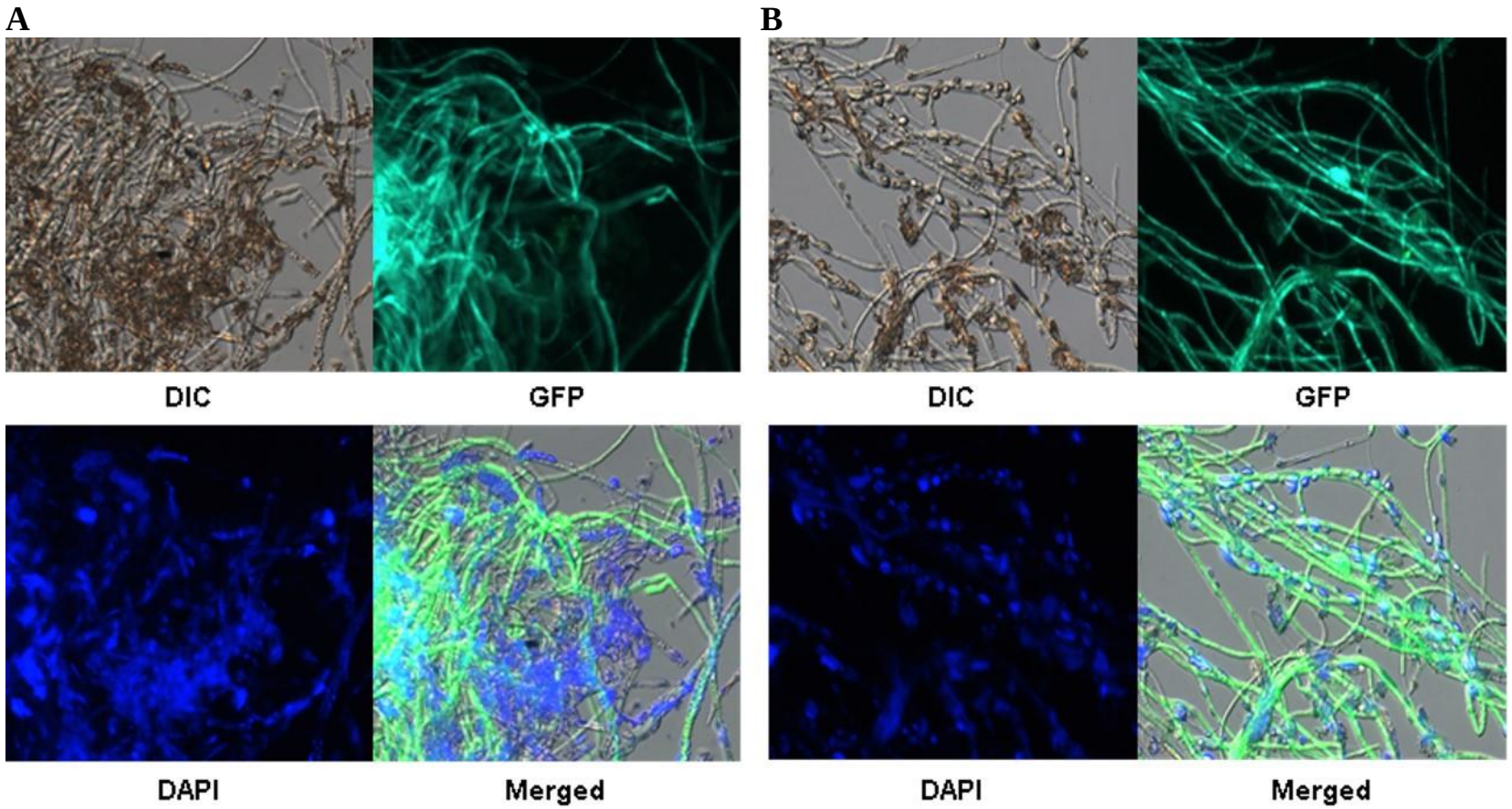
A
B
DIC
GFP
DIC
GFP
DAPI
Merged
DAPI
Merged

Fig. 1. Localization of Pks4 and Pks13 in Zeb2OE strains. A) Green fluorescent protein (GFP) was tagged Pks4 and B) GFP was tagged Pks13. Zearalenone will appear under 365 nm ultraviolet light as blue fluorescent spots. The photographs were taken 21 days after inoculation.

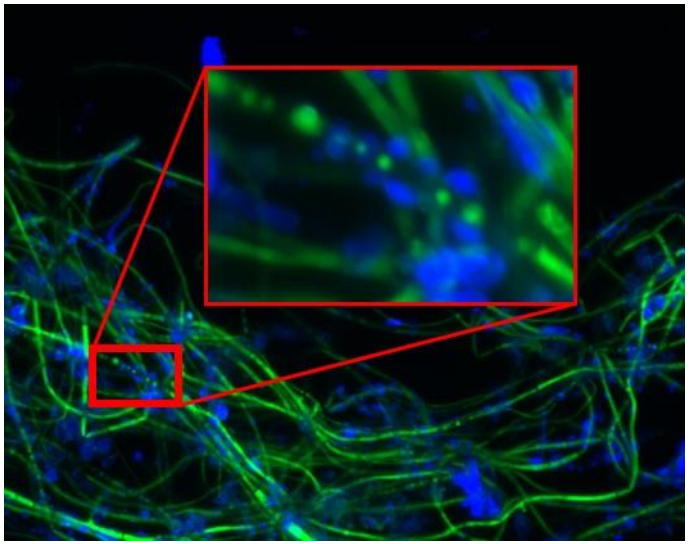

Fig 2. Localization of ZEA biosynthetic enzymes in Zeb2OE strain. In Zeb2OE strain, Pks-GPF usually localized to the cytoplasm, however a few Pks-GPF localized to the spherical structures in rice media. The photographs were taken 21 days after inoculation.

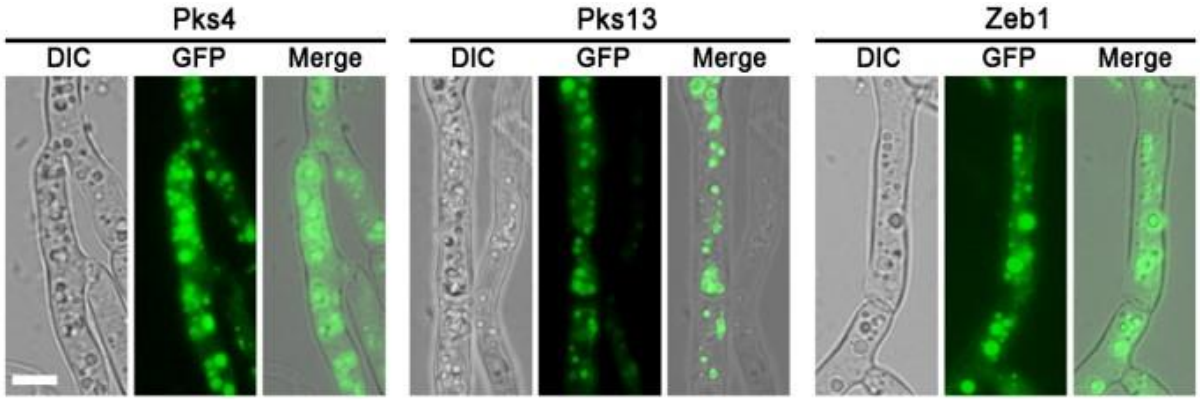

Fig. 3. Localization of ZEA biosynthetic enzymes. GFP was tagged at each ZEA biosynthetic enzymes. In wild type strains, Pks4-GFP, Pks13-GFP, and Zeb1-GFP localized to the spherical structures of mycelium. The photographs were taken 4 days after inoculation. Scale bar = 10 μm.

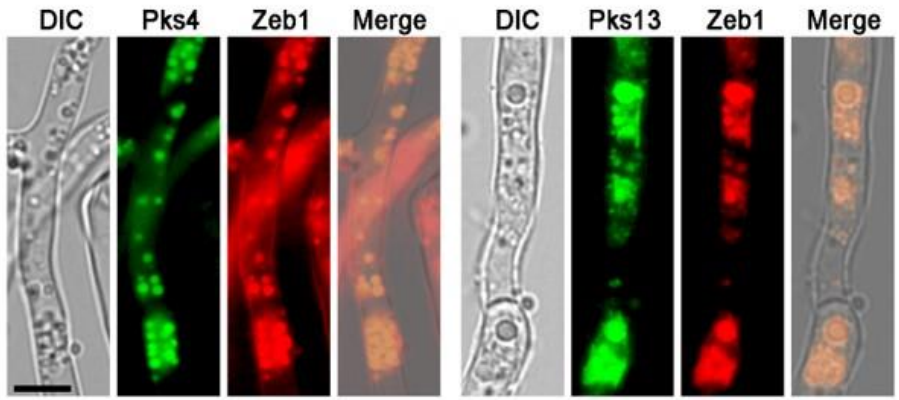

Fig 4. Co-localization of ZEA biosynthetic enzymes. Zeb1-RFP proteins were co-localized with Pks4-GFP or Pks13-GFP in hyphae grown in SG media. The photographs were taken 4 days after ZEA induction. Scale bar = 5 μm.

Cellular compartmentalization of ZEA biosynthetic enzymes

Since all ZEA biosynthetic enzyme was observed to localized in the same location, FM4-64, which is used to stain membrane, was utilized to observe which cellular organization the enzyme was expressed in. The results showed that most of cellular compartments stained with FM4-64 was overlapped with GFP fluorescence (Fig. 5)

Since FM4-64 had difficulty in identifying the exact organelles of the cell membrane and I supposed that ZEA is produced in peroxisomes, RFP tagged with the peptide sequence SKL, which specifically recognizes peroxisome, was expressed in Pks4-GFP and Pks13-GFP strains resulting in Pks4-GFP::SKL-RFP and Pks13-GFP::SKL-RFP, respectively. Most GFP signals were overlapped with RFP fluorescence suggesting that ZEA biosynthetic enzymes localized in peroxisomes during ZEA biosynthesis (Fig. 6).

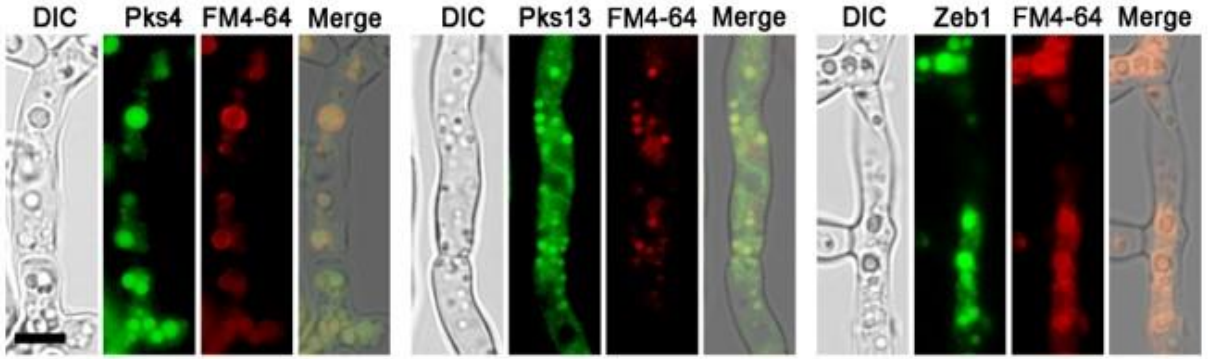

Fig. 5. Localization analysis of ZEA biosynthetic enzymes with FM4-64 stain. After growth in SG media, hyphae of Pks4-GFP, Pks13-GFP and Zeb1-GFP strains were stained with FM4-64 and examined for GFP and FM4-64 signals. The photographs were taken 4 days after ZEA induction. Scale bar = 5 μm.

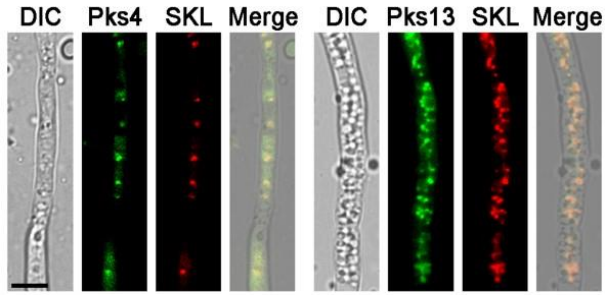

Fig. 6. Cellular compartmentalization of ZEA biosynthetic enzymes. SKL tagging at RFP was inserted in Pks4-GFP or Pks13-GFP strains. After growth in SG media for 1 days, hyphae of Pks4-GFP and Pks13-GFP strains inserted RFP-SKL examined for GFP and RFP signals. The photographs were taken 1 days after ZEA induction. Scale bar = 5 μm.

DISCUSSION

Previous studies have shown that secondary metabolites of the polyketide family, such as aflatoxin, trichothecene, and melanin, are isolated and ejected by toxisome (Kenne et al., 2014; Kistler and Broz, 2015) because these secondary metabolites are toxic to cells that produce them (Kistler and Broz, 2015; Feng *et al.*, 2020). Also, melanin is produced in toxisome derived from endosome, aflatoxin is derived from peroxisome, and trichothercenes is derived from vacuole (Chen *et al.*, 2021).

In this study, GFP was first observed by tagging ZEA biosynthetic genes Pks4 and Pks13 and Zeb1 to ensure that ZEA is isolated and synthesized like other secondary metabolites. However, when first observed in GFP tagging mutants based on wild type strain, the fluorescence was not clearly expressed. Therefore we constructed the GFP tagging mutants based on Zeb2OE strain, which is over-expressing ZEA biosynthetic genes. However, the observation showed rather excessive fluorescence (Fig. 1). Since ZEA was known to have a lower half-lethal dose compared to other mycotoxins, it was thought that ZEA was expressed throughout the cytoplasm irrespective of other secondary metabolites. However, through repeated observation (Fig. 2), it was confirmed that it was observed only in a part of the mycelium. For more detailed observation, GFP-tagged mutants

were prepared based on the wild-type strain again and inoculated into the SG media, which induces ZEA synthesis. As a result, green fluorescence was observed in the spherical structures that are presumed to be toxisome, just like other secondary metabolites (Fig. 3).

Aflatoxin and trichothecenes are synthesized through the action of multiple proteins at multiple sites (Kenne et al., 2014). However, since ZEA has a simple synthesis mechanism, there was a possibility that it could be synthesized in the same place. Therefore, in this study, based on mutants tagged with GFP on Pks4 and Pks13, mutants tagged with RFP on Zeb1 were constructed and observed. Consequently, it was observed that Pks4, Pks13, and Zeb1 were all located in the same place (Fig. 4).

After confirming that all of the ZEA biosynthetic enzymes are located in the spherical structures, FM4-64 was first stained with mutants in which GFP was tagged with ZEA biosynthetic enzymes to identify the corresponding organelle. In consequence, it was confirmed that staining was performed in all places where fluorescence was expressed (Fig. 5). Therefore, it could be confirmed that the organelle was one of the organelles generated by the separation of the cell membrane.

In order to confirm exactly the corresponding organelle, RFP tagging with SKL, a tripeptide sequence that targets and recognizes peroxisome, was randomly inserted into the mutants tagged with GFP to Pks4 and Pks13, followed by observation under a fluorescence microscope. As a result, it

was observed that red fluorescence was expressed where green fluorescence was expressed (Fig. 6). Therefore, it was confirmed that the place where the ZEA biosynthetic enzymes are located is the peroxisome.

In this study, it was confirmed that the place where the ZEA biosynthetic enzymes are located is the peroxisome. In future research, it is expected that ZEA biosynthetic enzymes will be able to gain a deeper understanding by confirming whether they are properly expressed when located in the cell nucleus. In addition, the endoplasmic reticulum (ER) is involved in the synthesis of melanin, and if we study whether ZEA is also involved in the synthesis of ER, it is expected that we will have a deeper understanding of the synthesis of mycotoxins and secondary metabolites.

LITTERATURE CITED

Bowden, R. L., & Leslie, J. F. (1999). Sexual recombination in *Gibberella zeae*. *Phytopathology, 89*(2), 182-188.

Chanda, A., Roze, L. V., Kang, S., Artymovich, K. A., Hicks, G. R., Raikhel, N. V., . . . Linz, J. E. (2009). A key role for vesicles in fungal secondary metabolism. *Proceedings of the National Academy of Sciences, 106*(46), 19533-19538.

Chen, X., Zhu, C., Na, Y., Ren, D., Zhang, C., He, Y., . . . Jiang, Y. (2021). Compartmentalization of Melanin Biosynthetic Enzymes Contributes to Self-Defense against Intermediate Compound Scytalone in *Botrytis cinerea*. *Mbio, 12*(2).

Desjardins, A., & Proctor, R. (2007). Molecular biology of *Fusarium* mycotoxins. *International journal of food microbiology, 119*(1-2), 47-50.

Desjardins, A. E. (2006). *Fusarium mycotoxins: chemistry, genetics, and biology*: American Phytopathological Society (APS Press).

Feng, N., Wang, B., Cai, P., Zheng, W., Zou, H., Gu, J., . . . Bian, J. (2020). ZEA-induced autophagy in TM4 cells was mediated by the release of Ca2+ activates CaMKKβ-AMPK signaling pathway in the endoplasmic reticulum. *Toxicology letters, 323*, 1-9.

Fischer-Parton, S., Parton, R., Hickey, P., Dijksterhuis, J., Atkinson, H., & Read, N. (2000). Confocal microscopy of FM4-64 as a tool for

analysing endocytosis and vesicle trafficking in living fungal hyphae. *Journal of microscopy, 198*(3), 246-259.

Gaffoor, I., & Trail, F. (2006). Characterization of two polyketide synthase genes involved in zearalenone biosynthesis in *Gibberella zeae*. *Applied and Environmental Microbiology, 72*(3), 1793-1799.

Goswami, R. S., & Kistler, H. C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular plant pathology, 5*(6), 515-525.

Kenne, G. J., Chakraborty, P., & Chanda, A. (2014). Modeling toxisome protrusions in filamentous fungi. *JSM Environ Sci Ecol, 2*, 1010-1012.

Kenne, G. J., Gummadidala, P. M., Omebeyinje, M. H., Mondal, A. M., Bett, D. K., McFadden, S., . . . Mitra, C. (2018). Activation of aflatoxin biosynthesis alleviates total ROS in *Aspergillus parasiticus*. *Toxins, 10*(2), 57.

Kim, Y. T., Lee, Y. R., Jin, J., Han, K. H., Kim, H., Kim, J. C., . . . Lee, Y. W. (2005). Two different polyketide synthase genes are required for synthesis of zearalenone in *Gibberella zeae*. *Molecular microbiology, 58*(4), 1102-1113.

Kistler, H. C., & Broz, K. (2015). Cellular compartmentalization of secondary metabolism. *Frontiers in Microbiology, 6*, 68.

Lee, S., Son, H., Lee, J., Lee, Y.-R., & Lee, Y.-W. (2011). A putative ABC

transporter gene, ZRA1, is required for zearalenone production in *Gibberella zeae*. *Current genetics, 57*(5), 343-351.

Leslie, J., & Summerell, B. (2006). The Fusarium laboratory manual. *The Fusarium laboratory manual.*

Lysøe, E., Klemsdal, S. S., Bone, K. R., Frandsen, R. J., Johansen, T., Thrane, U., & Giese, H. (2006). The *PKS4* gene of *Fusarium graminearum* is essential for zearalenone production. *Applied and Environmental Microbiology, 72*(6), 3924-3932.

Marasas, W. F. O., Nelson, P. E., & Toussoun, T. (1984). *Toxigenic Fusarium species. Identity and mycotoxicology*: Pennsylvania State University.

Min, K., Son, H., Lee, J., Choi, G. J., Kim, J.-C., & Lee, Y.-W. (2012). Peroxisome function is required for virulence and survival of *Fusarium graminearum*. *Molecular plant-microbe interactions, 25*(12), 1617-1627.

Mirocha, C., Pathre, S., Behrens, J., & Schauerhamer, B. (1978). Uterotropic activity of cis and trans isomers of zearalenone and zearalenol. *Applied and Environmental Microbiology, 35*(5), 986-987.

Park, A. R., Fu, M., Shin, J. Y., Son, H., & Lee, Y.-W. (2016). The protein kinase A pathway regulates zearalenone production by modulating alternative *ZEB2* transcription. *Journal of microbiology and*

biotechnology, 26(5), 967-974.

Russell, D. W., & Sambrook, J. (2001). *Molecular cloning: a laboratory manual* (Vol. 1): Cold Spring Harbor Laboratory Cold Spring Harbor, NY.

Shoji, J.-y., Arioka, M., & Kitamoto, K. (2006). Vacuolar membrane dynamics in the filamentous fungus *Aspergillus oryzae*. *Eukaryotic cell, 5*(2), 411-421.

Steele, J. A., Lieberman, J. R., & Mirocha, C. (1974). Biogenesis of zearalenone (F-2) by *Fusarium roseum*'Graminearum'. *Canadian journal of microbiology, 20*(4), 531-534.

Upadhyay, S., Xu, X., Lowry, D., Jackson, J. C., Roberson, R. W., & Lin, X. (2016). Subcellular compartmentalization and trafficking of the biosynthetic machinery for fungal melanin. *Cell reports, 14*(11), 2511-2518.

Windels, C. E. (2000). Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. *Phytopathology, 90*(1), 17-21.

Zinedine, A., Soriano, J. M., Molto, J. C., & Manes, J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food and chemical toxicology, 45*(1), 1-18.

요약 (국문초록)

지랄레논 생합성 효소의 세포 구획화 연구

전민호

Fusarium graminearum 은 전세계적으로 밀, 보리, 옥수수, 벼 등 주요 작물에 Fusarium head blight (FHB)를 일으키는 중요한 식물병원균이다. 이 곰팡이는 심각한 수확량 감소를 일으킬 뿐만 아니라 감염된 작물에 zearalenone (ZEA)과 trichothecene (DON and NIV) 등의 곰팡이독소를 축적시켜 인간과 동물의 건강에 심각한 위협을 끼친다. ZEA의 구조는 에스트로겐과 유사하며 포유류의 에스트로겐 수용체와 결합하여 에스트로겐의 작용을 방해하여 생식기능 장애를 일으킨다. 이전 연구에서는 ZEA 생합성 유전자 (*PKS4*, *PKS13*, *ZEB1*, *ZEB2*)와 그들의 *F. graminearum*에서 ZEA 생합성의 작용 메커니즘에 대해 연구하였다. 그러나 ZEA 생합성 효소의 세포내 위치와 ZEA 생산에 필요한 세포 조직에 대해서는 거의 알려져 있지 않다. 본 연구에서는 녹색형광단백질(GFP)을 Pks4, Pks13, Zeb1에

표지하였고, 모든 단백질이 ZEA 유도 조건에서 특정 세포소기관에 위치하는 것을 관찰하였다. Zeb1-적색 형광 단백질 (RFP) 결합 단백질을 Pks4-GFP와 Pks13-GFP 균주를 기반으로 하여 발현시켰다. 그 결과 Pks4, Pks13, Zeb1은 특정 세포소기관에서 위치하는 것을 보이고 있었다. 특정 세포소기관을 밝히기 위해 FM4-64 염색과, peroxisome 표적 신호인 SKL에 RFP를 표지하여 관찰하였다. 그 결과 모든 ZEA 생합성 효소가 퍼옥시좀(peroxisome)에 위치하는 것을 확인하였으며, 이는 zearalenone이 peroxisome에서 생합성됨을 시사한다. 본 연구는 polyketide 계열의 이차대사산물이 합성되는 위치에 대한 정보를 제공하며, 새로운 곰팡이 독소 저해 전략을 개발할 수 있을 것이라고 생각한다.

주요어 : 붉은곰팡이, Zearalenone(지랄레논), 이차대사산물, 세포내 구획화, Peroxisome(퍼옥시좀)

학 번 : 2019-26417