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The Past, Present, and Future of Genetic Manipulation in Human Fungal Pathogen *Talaromyces marneffei*

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The fungus *Talaromyces marneffei* has been discovered and its pathogenicity to humans has been recognized for over 60 years. The advances in organism-wide studies and the development of genetic manipulation tools contribute greatly to our current understanding of host-pathogen interactions. Several classes of genes have been identified to be involved in stress response, morphogenesis, and virulence based on the characterization of the generated mutants. Here, we summarize the main techniques for *T. marneffei* genetic manipulation, including chemical mutagenesis, insertional mutagenesis, homologous recombination-mediated gene replacement, knockdown methods, and the recent popular clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9 (CRISPR-Cas9) technology. The advantages and disadvantages of each technique were determined from a historical perspective. We also describe potential strategies to improve the current genetics studies, such as the generation of new selection markers and genetically modified strains. Our review has demonstrated that Thailand will continue to make efforts to become a leader in *T. marneffei* genetics research. The genetic approaches have impacted the studies of *T. marneffei* and can lead to the discovery of new diagnostic tools, drugs, and vaccines.

Keywords: Genetics manipulation, Talaromycosis, *Talaromyces marneffei*, CRISPR

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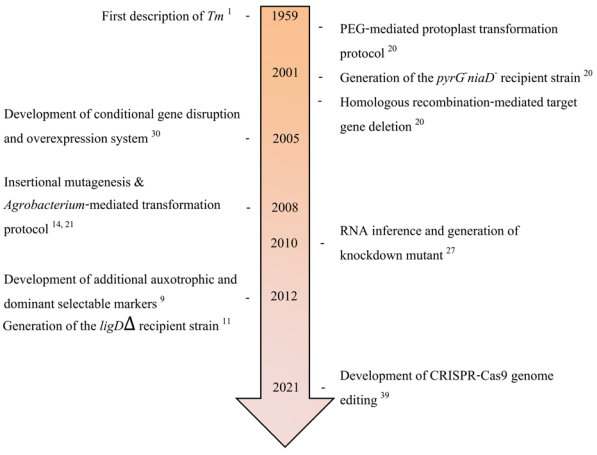
Talaromycosis: The Need for Better Therapeutic Interventions

Talaromyces marneffei can cause a potentially fatal systemic mycosis called talaromycosis in humans, particularly in immunocompromised individuals.[1] *T. marneffei* infection is endemic to tropical and subtropical Asia.[2-4] Over 17 300 cases have been reported annually,[5] and up to one-third of these infections are associated with death.[6] In northern Thailand, talaromycosis is the third most common AIDS-associated disease.[2, 7] Although the morbidity and mortality of talaromycosis are high, and *T. marneffei* is even listed as one of the fungal priority pathogens by the World Health Organization (WHO), the study of *T. marneffei* still has not received sufficient attention and investment from regional and global funders, researchers, and clinicians.[5, 8] As a result, the control and prevention of this life-threatening disease remains elusive.

T. marneffei is a thermally dimorphic fungus that grows as filamentous hyphae at environmental temperatures (25°C), and undergoes morphological switching to yeast form at human body temperature (37°C). The primary infection site is the lung, presumably via the inhalation of conidia from the environment. The conidia are engulfed by the alveolar macrophages, but they usually survive intracellularly among immunocompromised individuals. The fungus turns into a yeast growth phase inside the phagosome, divides by fission, and lyses the macrophages for dissemination.[3] In the first stage, lung infection results in nonspecific respiratory symptoms. Later, the fungus invades the blood circulation and mononuclear phagocyte system, causing multiorgan defective symptoms such as lymphadenopathy, hepatosplenomegaly, anemia, and skin lesions. Thus, the ability to switch morphologies and escape macrophage killing is necessary for *T. marneffei* to become a successful pathogen.

Identification of molecular pathways governing fitness and virulence traits in this organism can improve the understanding of how *T. marneffei* invades the host, survives the host defense mechanisms, and ultimately causes disease. Importantly, this knowledge can facilitate the development of biomarkers, diagnostic tools, and antifungal reagents. The ability to genetically manipulate the genome of *T. marneffei* is indispensable for dissecting gene functions at molecular levels. However, genetic studies in *T. marneffei* have been encumbered by multiple factors, including the limitation in selection markers, transformation methods, and gene editing techniques. This review summarizes the genetic manipulation of *T. marneffei* over 2 decades, starting from the year 2000 (Figure 1). We discuss potential strategies to improve available genetic tools, including the recently developed CRISPR-Cas systems.

Figure 1. Timeline of Key Genetic Modifications in *Talaromyces marneffei*



Abbreviations: CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9; PEG, polyethylene glycol; *Tm, Talaromyces marneffei.*

Selection Markers in *Talaromyces marneffei* Genetic Research

Both auxotrophic and dominant selection markers have been commonly used in *T. marneffei* genetic manipulation, and pioneer works have been developed by Andrianopoulos and colleagues.[9, 11] The complete list of selection markers available in *T. marneffei* ATCC18224 strain can be found in Boyce et al.[9] However, representative markers are shown in Table 1. Auxotrophic genes, defined as genes that encode an essential protein for biosynthesis of an essential nutrient, are widely used for selection of positive transformants for use in genetic engineering. Common auxotrophic selection markers used in *T. marneffei* are *pyrG* (encoding orotidine 5'-monophosphate decarboxylase), *niaD* (encoding nitrate reductase), *riboB* (encoding putative GTP cyclohydrolase for riboflavin biosynthesis), and *pyroA* (encoding a protein required for biosynthesis of pyridoxine).[9] The advantage of using auxotrophic selection markers is the affordability of selection compounds. Another advantage is that some auxotrophic markers, such as *pyrG* and *niaD* markers permit positive and negative selection.[9] Moreover, the *pyrG* blaster cassette is designed to permit removal and reuse of the selection marker. In the *pyrG* blaster, the *pyrG* gene is flanked with direct CAT repeat sequences and can be readily removed from the *T. marneffei* genome by recombination at CAT repeat sequences. However, in comparison to other fungal pathogens such as *Aspergillus fumigatus*, *Candida albicans*, or *Cryptococcus neoformans*, the auxotrophic markers are less available in *T. marneffei*, and are limited to only a few laboratory strains. Most of the available recipient strains have been generated in the ATCC18224 strain background.[10, 11]

Drug resistance genes are generally used as dominant selection markers. As opposed to auxotrophic selection markers, the use of dominant selection markers allows genetic manipulation in any of various genetic backgrounds without the need to generate the relevant recipient strains before genetic manipulation. However, dominant markers are selected on drug compounds, which could be expensive.

Table 1. Selection Marker Systems Used in Genetic Manipulation of *Talaromyces marneffei*

Selection Marker	Recipient Strain	Description (Plasmid)	Phenotype
Auxotrophic system			
pyrG	*pyrG*$^-$	*Aspergillus nidulans pyrG* blaster cassette (pAB4626)	- Uracil auxotroph - 5-Fluoroorotic acid resistant
niaD	*niaD*$^-$	*Aspergillus oryzae niaD*	- Nitrate utilization defect - Chlorate resistant
riboB	Δ*riboB*	*Aspergillus nidulans riboB* gene	Riboflavin auxotroph
pyroA	Δ*pyroA*	*Aspergillus nidulans pyroA* gene	Pyridoxine auxotroph
Dominance system			
hygR (hph)	Any	Hygromycin B resistance genes (pAN7.1)	Hygromycin B resistant[12-14]
BleoR	Any	Bleomycin resistance gene (pAN8.1)	Bleomycin resistant[12-14]
Phleomycin	Any	*Streptococcus hindustanus* phleomycin resistance gene (pAN8.1)	Phleomycin resistant[15]

In some clinically isolated strains, natural drug tolerance could occur, and hence might require a higher concentration of drugs for selection. Overall, there are sufficient selection markers to generate a single gene mutant and revertant strain. However, the limitations in selection marker systems and tedious steps in selection marker recycling hinder the genetic manipulation of multiple genes, and hence the study of genetic interactions in this fungal pathogen.

Perspectives

Although selection markers are available in *T. marneffei*, the development of new selection marker systems and the generation of laboratory strains in various genetic backgrounds are still urgently needed. For example, adenine auxotrophic marker gene, encoding enzyme phosphoribosyl aminoimidazole carboxylase of purine biosynthetic pathway, has been commonly used in several fungal species such as in *Saccharomyces cerevisiae* (*ADE2* gene), *C. albicans* (*ADE2* gene), and *A. oryzae* (*adeB* gene). Unlike other auxotrophic markers recently used in *T. marneffei* (*pyrG*, *niaD*, *riboB*, *pyroA*),[9] the adenine auxotrophic mutant will have red color colonies.[16] This is a result of polymerization and oxidation of an accumulated intermediate, 4-amino-imidazole ribotide, due to a blockade in the adenine biosynthetic pathway.[17] Complementation with functional *ade2* gene will reverse the red to white color colonies. Thus, the selection based on color can offer a new strategy for genetic studies or high-throughput screening experiments in *T. marneffei*.

Many phenotypes of *T. marneffei* exhibit strain background specificity.[18, 19] Thus, the generation of genetically modified strains in diverse backgrounds could be useful for comparative analyses. For instance, the *T. marneffei* strain ATCC200051 is clinically isolated from patients in Thailand. To allow genetic studies in this strain background, the *T. marneffei* strain ATCC200051 could be subjected to chemically induced mutagenesis to generate an uracil auxotroph (unable to grow on a medium without uracil). This genetically modified strain can then be established as another experimentally amenable system to study morphogenesis and pathobiology.

Strategy to Study Forward Genetics in *Talaromyces marneffei*

Forward genetics refers to the identification of the genetic basis that underlies a specific phenotype. Genetic screens are classical approaches, involved with randomly mutating the genome of the organism and then assessing the mutants for phenotypes of interest. In *T. marneffei*, chemical mutagenesis and random insertional mutagenesis have been successfully performed. In chemical mutagenesis, the screens are usually conducted for the purpose of identifying selection markers and generating recipient strains. For instance, the 5-fluoroorotic acid (5-FOA) resistant mutants and chlorate resistant mutants have been isolated.[11, 20] In addition to the chemical approach, the *Agrobacterium*-mediated transformation (AMT) has been developed as an efficient tool for random insertional mutagenesis.[12, 14, 21, 22] This AMT approach has been successfully performed to identify novel genes involved in morphogenesis and pigment production.[12, 14, 21]

Reverse Genetics Tools Available in *Talaromyces marneffei*

Reverse genetics are the study of phenotypic consequences after specific gene sequences are mutated. As the whole genome sequences of *T. marneffei* have been identified in multiple strain backgrounds,[23-25] reverse genetics have accelerated the understanding of *T. marneffei* biology and pathogenicity. In principle, DNA double-strand breaks (DSBs) can be repaired by either non-homologous end joining (NHEJ), or

the homology directed repair (HDR) pathways. The HDR pathway has been exploited in the reverse genetics approach to precisely target specific genetic locus and introduce desired sequence alterations through a homologous DNA template during the repair events. The targeted gene manipulation was initially created in *T. marneffei* by the Andrianopoulos laboratory to delete the *abaA* and *stlA* genes.[20, 26] As in other eukaryotes, *T. marneffei* prefers to repair DSBs via the NHEJ, decreasing the frequency of target gene modification via the homologous recombination during genetic manipulation. To enhance successful gene targeting, disruption of the *ligD* gene (*ligD*Δ recipient strain), encoding the DNA ligase component of a NHEJ pathway, markedly improved the success of homologous gene replacement in this fungus.[11] Even though removal of the *pkuA* gene, encoding the protein kinase component of the NHEJ, can increase the frequency of homologous integration of transforming DNA fragments, the *pkuA*Δ mutant was associated with reduced genetic stability and accumulated aberrant phenotypes over time.[11]

In addition to the complete gene knockout strategy, the RNA inference-mediated gene knockdown has been applied in *T. marneffei* research. The gene knockdown strategy is especially useful for systematically manipulating multiple genes within the same family. For example, secretory mannoproteins *(Mp1, Mplp1-Mplp13)* and pigment biosynthetic genes *(pks1 - pks25)* were systematically knocked down, leading to the discovery of key virulence factors during *T. marneffei* infection.[15, 27, 28]

Conditional Target Gene Expression and Overexpression System in *Talaromyces marneffei*

Conditional target gene expression and overexpression system is important to study the essential genes, defined as their requirement for functions and cannot be disrupted for the classical loss-of-function study. The gold standard strategy to study such genes is to generate the conditional mutant strain in which the target gene can be expressed at controllable levels and time. To construct conditional mutants, desired genes are engineered to express under inducible promoters. In *T. marneffei*, the *xylP* promoter from *Penicillium chrysogenum* has been characterized and used to modulate the expression of target genes.[29-31] Glucose is a strong repressor while sucrose and xylose are strong inducers of this *xylP* promoter, and the concentrations of repressing and inducing carbon sources determine the gene expression levels driven by this promoter. The *xylP* expression system has been successfully applied to study the essential TATA-binding protein (TBP) gene.[30] In addition to the study of essential genes, the inducible promoter and gene overexpression system has been used to characterize the function of genes, which their expression levels are highly upregulated in the pathogenic yeast form, such as the *msgA* gene.[31]

Furthermore, the transcription elongation factor alpha *(tefA)* is another promoter used in the *T. marneffei* gene overexpression approach. As opposed to the *xylP* inducible promoter, the gene regulated by the *tefA* promoter is constitutively expressed at high levels under all conditions because it is a housekeeping gene. Accordingly, the *tefA* promoter can be exploited to drive the expression of synthetic genes such as in the case of the CRISPR-Cas9 system.[29] Also, the *tefA* promoter has been successfully used to investigate the function of the *madsA* gene in regulating the *T. marneffei* phase transition.[30] The *madsA* gene is highly expressed during the yeast-to-mold transition and overexpression of the *madsA* under the *tefA* promoter induces mold growth at 37°C. Overall, the overexpression system has been developed in *T. marneffei*, and it is a powerful tool to investigate the role of essential genes and permits the gain-of-function analysis to complement the loss-of-function study.

CRISPR-Cas9 Mediated Genome Editing in *Talaromyces marneffei*

Even though the conventional homologous recombination-mediated gene targeting and editing has been widely used and efficiency for this strategy has been improved over the years,[11, 32] this approach is still a long and tedious process because it depends on random DSB events at the desired target site. Generation and selection of the desired mutants could take longer than 6 months. Excitingly, the CRISPR-Cas technology creates a different scenario for genetic manipulation. CRISPR-Cas is an RNA-guided adaptive immune system used by bacteria and archaea to defend against viral infections. By associating with guided RNA, Cas endonuclease is directed to a specific genomic target to make DSBs. To apply the CRISPR-Cas system to genome editing, the RNA-guided Cas systems can be engineered to cleave virtually any DNA sequences, and the subsequent DSBs can be exploited as a basis for site-specific mutagenesis. This is because DSBs are lethal to the cells, and to survive, cells must repair these breaks via NHEJ or HDR (see reverse genetics section). To employ homologous recombination for CRISPR-mediated genome editing, the cells can recombine target DNA by using (donor) DNA templates that contain homologous regions flanking any desired gene modifications.[33, 34] Thus, the generation of DSBs at specific sites by the CRISPR-Cas system tremendously increases the rate of homologous recombination and hence the efficiency of genome editing.[32] In summary, while conventional homologous recombination-mediated gene targeting has been widely used, it remains a time-consuming process relying on random DSB events. In contrast, CRISPR-Cas technology offers a more efficient approach. By utilizing RNA-guided Cas endonucleases, CRISPR-Cas enables precise targeting of genomic sites, inducing DSBs that prompt cell survival mechanisms like NHEJ or HDR. Integrating homologous recombination with CRISPR-Cas significantly accelerates genome editing by increasing the rate of targeted DSBs.

Since the first publication demonstrating functionality *in vitro*, the CRISPR-Cas systems have been engineered into a powerful gene-editing tool that has revolutionized genetic manipulation in diverse species.[35-38] The CRISPR-Cas9 genome editing system has been recently developed for *T. marneffei*.[39] The *sakA* gene was successfully targeted for error-prone mutagenesis by NHEJ repair with a 40% success rate.[39] This newly developed genetic tool is promising, and several strategies can be applied to improve the current CRISPR-Cas9 system (Table 2), as discussed below.

Table 2. Limitations on Current CRISPR-Cas9 Method and Potential Strategy for Improvement.

Attribute	Zhang et al[39]	Strategy
DSB-mediated specific gene targeting	+	Generation of random mutations at specific target site
Genome editing to desired sequences	-	Addition of template (donor DNA) containing desired specific sequences
Cloning-free gRNA assembly	-	Fusion PCR to generate the CRISPR-Cas cassettes
Markerless genome editing and removal of CRISPR components	ND	Development of marker removal and recycle CRISPR-Cas system

Abbreviations: +, strategy is presented; -, strategy is absent; CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein; DSB, DNA double-strand break; gRNA, guided RNA; ND, strategy is not determined; PCR, polymerase chain reaction.

Application and Perspectives

The specificity of RNA-guided CRISPR endonucleases (Cas proteins) is dependent on both the protospacer sequences in guided RNA and the protospacer adjacent motif (PAM) at the target site. Since gene editing with Cas9 is restricted by its 5'-NGG-3' PAM sequences, it is desirable to develop other Cas nucleases with different PAM sequences. This specific PAM sequence could be problematic for genetic manipulation in the-TA rich genome organisms. To overcome this problem, Cpf1 (also known as Cas12), employing a PAM sequence of 5'-TTTV-3', could become another alternative tool to expand the repertoire of CRISPR-mediated gene editing. The CRISPR-Cas12 system has been developed in multiple fungal species, such as in *Aspergillus* species.[40] It will become beneficial to have another CRISPR-Cas12 system for genetic manipulation in *T. marneffei*.

Importantly, the CRISPR technology can be conveniently used to manipulate genes on a large scale as demonstrated in many organisms. First, the CRISPR-based platform has been programed to edit multiple genes simultaneously, hence allowing the genetic interaction study.[40-43] Second, the CRISPR components have been combined with genome-scale guided RNA libraries for unbiased phenotype screening, hence permitting the forward genetics analyses.[44] Unlike traditional chemical or insertional mutagenesis methods that induce random mutations and require subsequent laborious identification of these modifications, the CRISPR system can create libraries of mutations at known sites in the genome.[44] To move forward, the CRISPR-Cas technology can be used in *T. marneffei* research to generate the mutant libraries for forward genetics studies, create desire mutations at specific sites for reverse genetics analyses, and target multiple gene modifications for genetic interaction analysis.

From Genetics Study to Clinical Applications

The exploration of genes in various pathways has paved the way for the development of clinical applications. For example, fungal siderophores are iron-chelating agents with potential therapeutic applications in the treatment of patients with iron-overloading diseases or facilitating the delivery of antimicrobial substances to specific pathogenic fungi. The *T. marneffei sreA* deletion strain has been engineered to enhance siderophore production.[45] The purified extracellular siderophore, coprogen B, could lower the labile iron pool (LIP) levels in iron-loaded hepatocellular carcinoma cells without toxicity to the tested cells. In addition, the optimum levels of coprogen B did not enhance the growth of normal microflora such as *C. albicans*. Since the main portal of iron excretion from the human body is through the intestine, this result potentially showed that it will not enhance the risk for candidiasis when used as the iron chelator. Thus, the coprogen B isolated from the *T. marneffei sreA* deletion strain could be potentially used as a new and safe iron-chelating agent with minimal side effects to the host.

Genetics analysis can guide the selection of specific genes for follow-up studies and other downstream applications. For instance, a systematic knockdown study of 13 mannoproteins (MP1, MPLP1 - 13) has revealed that only MP1 is a key virulence factor in *T. marneffei* PM1 strain.[15] Subsequent experiments have identified that MP1 mannoprotein binds and sequesters arachidonic acid, the key proinflammatory meditator, allowing *T. marneffei* to evade the host's innate immune defense.[46] The MP1 antigen detection tools have been developed and successfully used in the diagnosis of talaromycosis in several clinical settings.[47, 48] Moreover, the promising result of developing MP1 as a vaccine has been demonstrated in an animal study. Immunization of BALB/c mice with recombinant protein MP1 could

protect against infection against *T. marneffei* with 100% survival.[49] Overall, knowledge obtained from genetic studies can have a broad implication for fungal pathogenesis, diagnosis, treatment, and disease prevention.

Current Progress on *Talaromyces marneffei* Genetics Study in Thailand

Thailand is one of the hyperendemic regions of *T. marneffei*; talaromycosis is the third most common opportunistic infection after tuberculosis and cryptococcosis.[4] In Thailand, the understanding of *T. marneffei* gene functions at molecular levels mainly comes from Chiang Mai University. Specifically, the encoding catalase-peroxidase *cpeA*, was first deleted from *T. marneffei* genome in 2005.[50] Since then, many genes have been characterized using the forward and reverse genetic approaches (Table 3). Thus, the studies from Thailand have contributed significantly to the research area of *T. marneffei*.

Table 3. Gene Mutagenesis of *Talaromyces marneffei* in Thailand

Gene	Approach	Phenotype	References
Catalase-peroxidase *(cpeA)*	Reverse genetics	The *cpeA* mutant is sensitive to oxidative stress.	Pongpom et al[50]
TATA-binding protein *(tbp)*	Conditional disruption		Pongsunk et al[30]
- ARF GTPase activator (Glo3) - S-adenosylmethionine decarboxylase proenzyme - Transcription factor *(stuA)* - Vacuolar ATP synthase subunit c - DUF907 domain protein - Short-chain dehydrogenase - G protein alpha subunit *(gasC)* - DNA damage response protein Rtt109 - SAM domain protein - Protein kinase Yak1	Forward genetics	The mutants show morphology defects.	Kummasook et al[12] Kummasook et al[14]
Transcription factor *(yakA)*	Reverse genetics	The *yakA* mutant showed early conidial germination, increased chitin content and abnormal chitin distribution.	Suwunnakorn et al[51]
Transcription factor *(atfA)*	Reverse genetics	The *atfA* mutant is sensitive to oxidative stress.	Nimmanee et al[13]
Stress-activated kinase *(sakA)*	Reverse genetics	The *sakA* mutant is sensitive to oxidative stress.	Nimmanee et al[52]
Transcription factor *(rttA)*	Reverse genetics	The *rttA* mutant is sensitive to morphogenesis and stress response.	Suwunnakorn et al[51]
Laccase *(pbrB)*	Reverse genetics	The *pbrB* deletion mutant showed defective DHN-melanin synthesis led to brown conidia production.	Sapmak et al[54]

Table 3. Gene Mutagenesis of *Talaromyces marneffei* in Thailand (Continued)

Gene	Approach	Phenotype	References
Transcription factor *(yapA)*	Reverse genetics	The *yapA* mutant is sensitive to oxidative stress and decreases in macrophage survival.	Dankai et al[55]
Mannoprotein *(mplp6)*	Reverse genetics	The *mplp6p* mutant exhibits similar response to stress and macrophage killing as wild type.	Pongpom et al[56]
Transcription factor of gluconeogenesis *(acuK)*	Reverse genetics	The *acuK* mutant is unable to utilize gluconeogenic carbon sources. The *acuK* mutant shows growth defects under low iron conditions.	Amsri et al[57]
Transcription factors of siderophore biosynthesis *(sreA and hapX)*	Reverse genetics	The *sreA* mutant overproduces siderophore.	Amsri et al[45]
Heat shock protein 30 *(hsp30)*	Reverse genetics	The *hsp30* mutant is sensitive to oxidative stress.	Wangsanut et al[58]

Concluding Remarks

Genetic manipulation in *T. marneffei* has developed at a steady pace since the year 2000 and contributed significantly to an understanding of *T. marneffei*'s pathogenesis. Investigating genes in various pathways has led to clinical applications, such as using siderophores and specific mannoproteins in therapeutic and diagnostic purposes. The development of a selection marker system and the expansion of genetically modified strains can accelerate the exploration, understanding, and ultimately the development of better therapeutic agents. To continue being one of the main genetic research hubs, the genetically modified strain *T. marneffei* with Thailand origin should be generated and whole genome sequencing of this strain should be publicly available. As the CRISPR-based method can precisely generate DSB at specific target locus rather than being dependence on random DSB events in traditional methods, the improvement of the CRISPR system will allow for even more efficient genetic manipulation in the fungal research community. In summary, we believe that current attempts and future advances in the CRISPR-based technology are promising to revolutionize the study of *T. marneffei* and medical mycology.

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การจัดการทางพันธุกรรมในเชื้อราก่อโรคในมนุษย์ *Talaromyces marneffei* อดีต ปัจจุบัน และอนาคต

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การค้นพบเชื้อรา *Talaromyces marneffei* และการศึกษาด้านกลไกการก่อโรคในมนุษย์นั้น มีมายาวนานกว่า 60 ปี ความก้าวหน้าในวิทยาการด้านต่าง ๆ และการพัฒนาเครื่องมือวิจัยเพื่อการจัดการทางพันธุกรรมในเชื้อราดังกล่าวได้มีส่วนสำคัญให้เกิดความเข้าใจปฏิสัมพันธ์ระหว่างโฮสต์ (Host) และเชื้อก่อโรคตลอดมา การศึกษาลักษณะเชื้อกลายพันธุ์ที่สร้างขึ้นช่วยให้เกิดการค้นพบยีนหลากหลายที่เกี่ยวข้องกับการตอบสนองต่อภาวะเครียด การเปลี่ยนรูป และปัจจัยส่งเสริมความรุนแรงของเชื้อ บทความนี้ได้สรุปเทคนิคหลักในการจัดการด้านพันธุกรรมในเชื้อ *T. marneffei* ได้แก่ การทำให้เกิดกลายพันธุ์ด้วยสารเคมี (Chemical mutagenesis) การแทรกยีน (Insertional mutagenesis) การทำโฮโมโลกัสรีคอมบิเนชัน (Homologous recombination-mediated gene replacement) การทำน็อคดาวน์ (Knockdown) รวมถึงการใช้เทคนิค CRISPR-Cas9 (Clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9) ที่เพิ่งมีการรายงานเมื่อเร็ว ๆ นี้ นอกจากนี้ ยังได้กล่าวถึงข้อดีและข้อเสียของเทคนิคต่าง ๆ รวมถึงกลยุทธ์ที่สามารถนำมาประยุกต์ใช้เพื่อให้การศึกษาด้านจีเนติกส์ (Genetics) ก้าวหน้ายิ่งขึ้น เช่น การสร้างเครื่องหมายคัดเลือก (Selection markers) และการสร้างเชื้อดัดแปลงพันธุกรรมชนิดใหม่ บทความปริทัศน์นี้ยังได้แสดงการมีส่วนร่วมของนักวิจัยในประเทศไทยเพื่อการศึกษาด้านพันธุกรรมในเชื้อ *T. marneffei* อย่างต่อเนื่อง ทั้งนี้ การศึกษาด้านจีเนติกส์สามารถสร้างผลกระทบให้เกิดการนำไปสู่การค้นพบวิธีใหม่ในการตรวจโรค การค้นพบยา และวัคซีนได้อย่างมีประสิทธิภาพต่อไป

คำสำคัญ: การจัดการทางพันธุกรรม โรค *Talaromycosis* เชื้อ *Talaromyces marneffei* เทคนิค CRISPR

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