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RESEARCH ARTICLE

Whole-Genome Sequencing of *Penicillium georgiense*, a Member of the Family Trichocomaceae Isolated from Harumanis Mango (Mangifera Indica L.)

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ABSTRACT - Microbial communities are present in various environments, influencing their hosts through both advantageous and pathogenic interactions. The advent of next-generation sequencing technologies has enabled more comprehensive exploration of these complex communities. *Penicillium georgiense* (*P. georgiense*), a known pathogenic fungus, has been shown to cause significant spoilage through rapid colonization enabled by spore production, particularly affecting long-term storage. Contamination by *Penicillium* species poses risks to crops health, emphasizing the need for effective post-harvest management. In this study, Harumanis mango, widely consumed in Asia, especially in Malaysia, was selected as the model host. A single colony of *P. georgiense* was isolated, and their genomic DNA was extracted. The genome of the *P. georgiense* H_A strain was sequenced using the Illumina NovaSeq 6000 platform with short-read technology, revealing a genome size of 18,324,307 base pairs and a GC content of 46.2%. BUSCO analysis indicated 98% genome completeness, with high similarity to the *P. georgiense* SRG7 isolate. The annotated genome has been deposited in the NCBI GenBank database for accessibility. This study provides important genomic insights into *P. georgiense*, advancing our understanding of its pathogenic mechanisms and offering valuable data for improved post-harvest management of Harumanis mango.

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1. INTRODUCTION

Penicillium, one of the largest and most widespread fungal genera known for their ability to thrive in various environments, such as soil [1], decaying organic matter [2], animal gut [3], and stored agricultural products, due to their exceptional adaptability. Due to their high survival ability in the nature, these species can have both positive and negative impacts on plants. Positively, *Penicillium* can support plant growth by improving nutrient absorption, producing phytohormones, boosting resistance to abiotic stress, and protecting against pathogens through competition for resources and the production of antibiotics and enzymes [4]. However, they also possess pathogenic traits that enable them to infect plants and cause diseases, such as producing enzymes like cellulase and proteases to evade plant cell wall, toxins (mycoxin and phytotoxin), as well as structural adaptations like hyphal growth, contributing to crop spoilage and significant economic losses in agriculture [5].

P. georgiense was first isolated from soil in peanut fields in Georgia, USA, and later to be found in sandy beach soil on Penang Island, Malaysia [6]. *P. georgiense* was isolated from diseased onion bulbs, where its pathogenic potential was demonstrated by the formation of weak yellow halos or yellowish, sunken lesions on inoculated bulbs [7]. Subsequently, another study reported the isolation of *P. georgiense* from decayed table grapes and examined its ability to induce decay in grape berries indicating the potential pathogenicity feature of the species [8]. Hence, this raises concerns about how this fungal species can infect crops [9], leading to spoilage and indirectly impacting food storage management [10].

The mango (*Mangifera indica L.*), often referred to as the "king of fruits," belongs to the Anacardiaceae family of dicotyledons [11]. It is rich in nutrients and known for its appealing flavor, delightful aroma, and enjoyable taste. The Harumanis mango is especially popular among Malaysians and other Asians. This variety is believed to have originated in Southeast Asia, where over 1,000 different cultivars have been identified globally. Harumanis, a specific variant of *Mangifera indica L.*, is primarily sourced from Indonesia and the northern regions of Malaysia [12]. Quality variation in mangoes can be influenced by various pre-harvest and post-harvest factors, leading to inconsistencies in fruit size, nutrient content, flavor, minerals, vitamins, and overall supply chain management, all of which also affect post-harvest handling practices [13].

Advancements in next-generation sequencing (NGS) have significantly expanded the scope of microbiology, enabling comprehensive studies of bacteria [14]–[18], fungi [19], [20], viruses [21]–[23], and their interactions within complex ecosystems. NGS of fungal species like *Penicillium* will significantly enhance our understanding of fungal diversity and

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their role in food storage, especially for vital crops such as mango. This technological advancement will enable the identification of fungi responsible for post-harvest infections, facilitating more targeted management strategies to reduce spoilage. By uncovering the pathogenic mechanisms of fungi like *P. georgiense*, which have been linked to crop damage and economic losses, we can develop improved storage and handling solutions. Ultimately, integrating NGS into agricultural practices will boost yields, minimize losses due to fungal contamination, and enhance both post-harvest management and sustainability, improving the overall quality of Harumanis mangoes.

2. METHODS AND MATERIAL

2.1 Isolation of Microbe and Growth Condition

A microbial community was cultured from the surface of decayed Harumanis mango, leading to the isolation of *Penicillium* species into a single colony via multiple sub-culturing steps. The incubation temperature was maintained at 30°C to promote optimal growth of *Penicillium* on Sabouraud Dextrose Agar (SDA) plates, with incubation lasting for four to five days.

2.2 DNA Extraction

Penicillium sp. genomic DNA (gDNA) was extracted using the FinePure Universal Genomic DNA Extraction Kit (GENFINE, Beijing, China), with some modifications to the manufacturer's recommended approach [24]. Before library preparation, the concentration and quality of gDNA were evaluated using a Qubit 4 Fluorometer (Thermo Scientific, USA) and agarose gel electrophoresis, respectively.

2.3 Sanger Library Preparation and Sequencing

ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA -3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3') primers were used to amplify the ITS region of the fungal genome. For the amplification process, reference PCR protocols were used and run in an Eppendorf Thermal Cycler: 5 minutes at 94 °C, followed by 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute, with a final extension period of 5 minutes at 72 °C [20]. For quality control, the concentration of the PCR product was measured using a Qubit 4 Fluorometer (Thermo Scientific, USA), and its quality was evaluated by agarose gel electrophoresis [25]-[26].

2.4 Whole Genome Library Preparation and Sequencing

Approximately 100 ng of DNA was fragmented to 350 bp using a Bioruptor, then Illumina library preparation was performed using the NEB Ultra II DNA library preparation kit (Thermo Scientific, US). The Illumina NovaSEQ6000 (Illumina, San Diego, CA) was used to sequence the samples, generating roughly 1GB of paired-end data (2x150 bp). The raw data will be processed and assembled for downstream application. The genome completeness was evaluated using the fungi odb10 databases and Benchmarking Universal Single-Copy Orthologue (BUSCO) version 5.2.1, using fungi_odb10 databases [26].

2.5 Genomic Analysis of *P. Georgiense*

The RASTtk scheme from the Rapid Annotation using Subsystem Technology (RAST) service was used to annotate the genome [27]. BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html) was used to assess the correctness of DNA sequence alignments. Using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/BLAST), the sequences retrieved for each strand were aligned and compared with sequences published in the GenBank database.

2.6 Data Availability

The complete genome sequence of *P. georgiense* was submitted to NCBI database as Bioproject, Biosample and Sequence Read Archive (SRA) with accession numbers PRJNA838218, SAMN28409049 and SRR19214155, respectively (http://www.ncbi.nlm.nih.gov/BLAST).

3. RESULTS AND DISCUSSION

In this study, *P. georgiense* was isolated as a single colony from a microbial community associated with decaying Harumanis mango. The ITS region sequence of *P. georgiense* was obtained through Sanger sequencing and subsequently aligned using the NCBI's Basic Local Alignment Search Tool (BLAST) to compare it with known sequences in the database. The results revealed a 99.15% identity with the SRG7 small subunit ribosomal RNA gene from *P. georgiense* isolates, confirming that the isolated fungus corresponds to the reference sequence. Subsequently, the Rapid Annotations using Subsystems Technology (RAST) server was utilized to annotate the complete genome of *P. georgiense*. The annotated genome measures approximately 18,324,307 bp, exhibits a G+C content of 46.2%, and contains a total of 348 RNAs. A summary of the genome characteristics is provided in Table 1.

Table 1. General feature of *P. georgiense* H_A strain

Attribute	Description
Genome size (bp)	18,324,307
Genome G + C content	46.2 %
Coding sequences	13775
Number of RNAs	348
N50	60
Number of contigs (PEGs)	637

Figure 1 shows that the RAST server-based annotation of the *P. georgiense* genome yielded 1173 protein-coding sequences, with 28 percent subsystem coverage. 3403 genes were found to produce functional proteins, with the remaining 104 genes serving as hypothetical proteins. The distribution of the genes in each subsystem feature had been assigned in numerous categories, revealing 859 genes involved in protein metabolism, followed by amino acids and derivatives (723), carbohydrates (610), cofactors, vitamins, prosthetic groups, pigments (547), RNA metabolism (434), stress response (252), fatty acids, lipids, and isoprenoids (243), DNA metabolism (230), nucleosides and nucleotides (226), respiration (202), virulence, disease and defense (193), cell wall and capsule (151), membrane transport (131), motility and chemotaxis (110), sulfur metabolism (102), regulation and cell signaling (95), miscellaneous (87), cell division and cell cycle (78), phosphorus metabolism (60), iron acquisition and metabolism (28), metabolism of aromatic compounds (26), nitrogen metabolism (23), phages, prophages, transposable elements, plasmids (22) and secondary metabolism (16), potassium metabolism (12) and dormancy and sporulation (3).

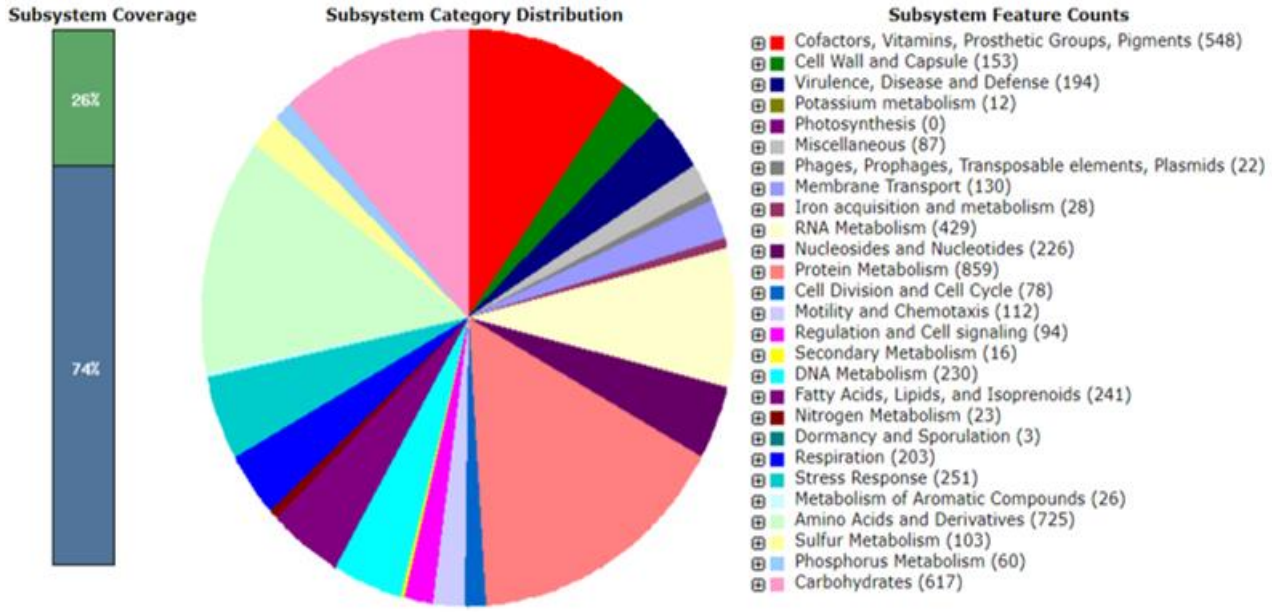

Figure 1. Distribution of gene in RASTk subsystem

In exploring the pathogenicity features of *P. georgiense*, we focus on two subcategories which were iron acquisition and metabolism and amino acids and their derivatives based on the annotated genes. Firstly, for iron uptake system, iron is particularly important in fungal pathogenesis as it serves as an essential cofactor for various metabolic processes necessary for the survival of both plants and pathogens, resulting in an intricate interplay in which plants employ strategies such as iron withholding and accumulation for defense, while pathogens develop mechanisms to counteract iron stress and manipulate host iron homeostasis to ensure their virulence during infection [28]. Thus, the ability of *P. georgiense* to acquire and utilize iron is pivotal to its pathogenicity. In fungal pathogenesis, iron acquisition occurs through two distinct mechanisms: siderophore-mediated iron uptake, which relies on the secretion of ferric iron-specific siderophores that bind to ferric ions (Fe^{3+}) in the environment, and the reductive iron uptake system, where cell wall iron reductases convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}) for direct cellular absorption [29]. Examining the annotated genes of *P. georgiense* in relation to the iron uptake system reveals several mechanisms at play, including the low pH-induced ferrous iron transporter EfeUOB, which comprises three genes, and the hemin transport system, which includes 19 genes. Although there is an absence of annotated genes for siderophores, this may suggest that *P. georgiense* employs a non-siderophore-dependent iron uptake system; however, further investigation is needed to validate the finding.

Moreover, for the amino acids and their derivatives subcategory, several annotated genes have been identified in *P. georgiense* that are involved in the metabolism of various protein compounds. Amino acids are fundamental to fungal pathogenesis in plants, as they not only provide essential nutrients for fungal growth and virulence but also act as building blocks for protein synthesis. Moreover, they participate in a wide range of metabolic pathways that are critical for fungal

survival, reproduction, and adaptation within the host environment [30]. The importance of these processes becomes evident during infection, where fungi must overcome plant defences and secure nutrients to thrive. Several amino acids, as shown in Table 2, have been specifically identified for their roles in nutrient acquisition and metabolism during fungal pathogenesis [31]. Based on RAST annotation, *P. georgiense* is predicted to possess the ability to metabolize certain amino acids that may enhance its pathogenicity toward plants, including arginine, lysine, threonine, methionine, histidine, glutamine, glutamate, aspartate, asparagine, proline, alanine, serine, glycine, and branched-chain amino acids such as valine and leucine. These amino acids could assist *P. georgiense* securing essential nutrients for survival and provide a competitive advantage in sustaining itself against other organisms within the host. Hence, the ability to synthesize or acquire specific amino acids under hostile conditions may contribute to *P. georgiense*'s virulence properties, allowing it to establish a more successful infection within plant tissues. A summary of the genome characteristics is provided in Table 2.

Table 2. Role of amino acids in fungal pathogenesis

Amino acids	Function in fungal pathogenesis
Glutamine	Serves as a nitrogen source and supports nitrogen metabolism, allowing fungi to utilize it for protein synthesis and various metabolic processes.
Asparagine	Acts as a nitrogen source and participates in amino acid metabolism, potentially playing a role in synthesizing other amino acids.
Methionine	Involved in sulfur metabolism and protein synthesis; fungi may need to biosynthesize methionine during infection due to its limited availability from the host.
Arginine	Essential for protein synthesis and serves as a precursor for molecules like nitric oxide, which are important for signaling during pathogenesis.
Branched-chain amino acids (leucine and valine)	Important for protein synthesis and may influence signaling pathways that regulate growth and metabolism in fungal pathogens.
Proline	Involved in stress responses and osmoregulation, assisting fungi in adapting to the host environment during infection.
Histidine	Important for protein synthesis and enzyme function; fungi may need to synthesize histidine during infection due to potential deficiencies in host tissues.
Alanine	Functions in protein synthesis and energy metabolism during fungal infection due to its availability in apoplast.
Aspartate	Contributes to the synthesis of other amino acids and metabolic pathways, potentially serving as a nitrogen source for fungi.
Glutamate	Important for protein synthesis and nitrogen metabolism during fungal infection due to its availability in apoplast.
Glycine	Involved in protein synthesis and metabolic processes to support fungal growth.

4. CONCLUSION

In summary, the complete whole genome sequence of *P. georgiense* provided valuable insights into its genetic potential. This data significantly enhances our understanding of *P. georgiense* as a pathogenic agent and its role in disease causation. These findings can be further leveraged in future studies to explore the presence and impact of *P. georgiense* in fruit contamination and spoilage in post-harvest management.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

H. F. Ahmad (Conceptualization; Formal analysis; Visualisation; Funding acquisition; Project administration; Supervision)

M. S. I. Sahran (Methodology; Data curation; Writing - original draft; Resources)

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