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Wickerhamomyces anomalus, a Biotechnological Yeast... an Opportunistic Pathogen?

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INTRODUCTION

Wickerhamomyces anomalus is a yeast with significant biotechnological potential, utilized in the production of fermented beverages and ethanol, as well as in the biocontrol of postharvest diseases, bioremediation of metals and organic contaminants, and surfactant production, among other applications. Its utility arises from its capacity to thrive on various carbon sources, tolerate a wide range of pH values, and survive temperatures ranging from 20 to 40°C (1). Nevertheless, documented cases of fungal infections in immunocompromised patients, including infants and adults, suggest its potential as an opportunistic pathogen(2). In our laboratory, we isolated a strain of *Wickerhamomyces anomalus* with potential for hexavalent chromium bioremediation (3). The aim of this study was to in silico assess the presence of virulence factor genes in the *Wickerhamomyces anomalus* M10 strain, which could confer opportunistic pathogenicity in humans.

MATERIALS AND METHODS

Genome Sequencing

DNA from *Wickerhamomhyces anomalus* M10 were sent to Macrogen for sequencing using the Illumina platform (NovaSeq, 150PE). The yeast genome size is 14.2 Mb.

Raw Data Processing

Read quality was assessed using FastQC, and reads were processed using Trimommatic to remove adapters while adhering to a Phred score of 20 as the quality threshold for the removal of low-quality reads.

Genome Assembly

Whole-genome assembly was performed using the Redundans pipeline, leveraging a reference genome obtained from JGI-Mycocosm. Assembly quality was assessed using QUAST.

Structural Annotation

Gene prediction was carried out using the FUNgap pipeline, which incorporates the BUSCO tool for quality assessment.

Functional Annotation

We employed the PHI-base (Pathogen-Host Interaction) database, which contains genes associated with virulence factors and provides essential information such as a PHI code, the pathogenic microorganism, and the host. Furthermore, these genes are categorized based on the phenotype changes related to virulence and pathogenicity when mutated. A blast search was conducted against this database, and the results were filtered with criteria that included a minimum identity of 70% and host organisms specified as humans or mice.

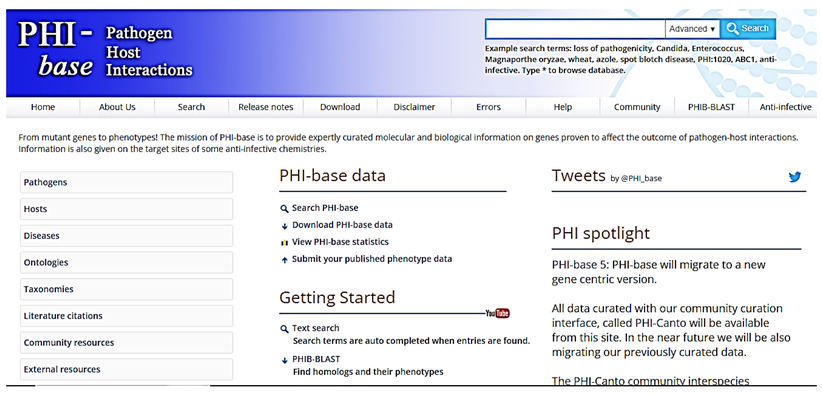

Image 1. PHI-base interface main

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RESULTS

Table 1. Genome assembly statistics**.**

Contigs	411
Contigs (>=0 bp)	703
Contigs (>= 1000 bp)	335
Contigs (>= 5000 bp)	80
Contigs (>= 10000 bp)	24
Contigs (>= 25000 bp)	10
Contigs (>= 50000 bp)	9
Largest contig	3 214 346
Total length	14 343 505
Total length (>=0 bp)	14 438 866
Total length (>=1000 bp)	14 288 948
Total length (>=5000 bp)	13 664 680
Total length (>=10000 bp)	13 272 614
Total length (>=25000 bp)	13 089 063
Total length (>=50000 bp)	13 060 986
N50	1 973 147
N90	455 794
auN	1 847 581
L50	3
L90	8
GC (%)	34.5
Mismatches	
N´s per 100 Kpb	1068.5
N´s	153 260

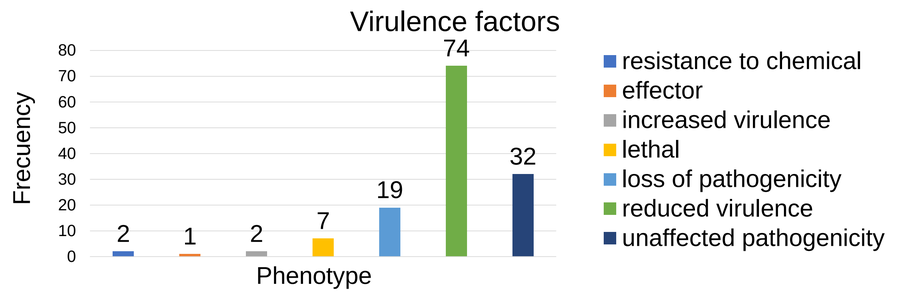
Table 2. Genome annotation attributes

Attributes	Values
Total protein-coding genes	7118
Transcript length (avg/med)	1,382.8/1,165.0
CDS length (avg/med)	1,350.5/1,140.0
Protein length (avg/med)	450.2/380.0
Exon length (avg/med)	1020.0-813.0
Intron length (avg/med)	99.3/57.0
Spliced genes	1656 (23.26%)
Gene density (genes/mb)	492.98
Number of introns	2,307
Number of introns per genes (med)	1.0
Number of exons	9,425
Number of exons per genes (med)	1.0

A total of 7118 protein-coding genes were identified.

Assembly quality. The assembly demonstrates exceptional quality, characterized by a notably high N50 value, a total length consistent with the genome size, and the presence of a limited number of contigs

Graphic 1. Identified Virulence factors with PHI-base



Identified virulence factors. Most of the identified genes were associated with the 'reduced virulence' phenotype, with a total of 74 genes found.

Table 3. identified functions of virulence factors

Reduced virulence		
Genes	Function	Affected phenotype
Arf2, CaRSR1, CaTUP1	membrane transport, cellular morphogenesis, Transcription factor	Hyphae formation
BMH1, CPAR2	Modulator protein, Transcriptional activator	Biofilm formation and interaction with immune cells
CST20, SIT4, SSN6	Protein kinase, Protein phosphatase, Transcriptional corepressor	Hyphae formation
MNN10	alpha-mannosyltransferase	Interaction with immune cells
Loss of pathogenicity		
Cas5, RFG1	Transcriptional regulator	Hyphae formation
CDC42	polarity establishment protein	Hyphae formation
CEK1	Mitogen-Activated Protein Kinase (MAPK)	Hyphae formation
CPH1__Cph1	Regulatory protein (hyphae formation)	Hyphae formation
RHO1	GTP-binding protein	Hyphae formation
VMA4	Vacuolar H+-ATPase complex	Hyphae formation

The genes identified with the 'reduced virulence' phenotype are involved in pseudohyphae formation, biofilm development, and interaction with host immune cells. On the other hand, genes identified with the 'loss of pathogenicity' phenotype are primarily associated with pseudohyphae formation. This demonstrates the yeast's potential for host invasion, which is a key aspect of virulence.

CONCLUSIONS

Based on this initial bioinformatic analysis of the *Wickerhamomyces anomalus* genome, we suggest that the yeast possesses genes that could facilitate its invasion and dissemination within the host, along with the ability to evade host defenses, potentially conferring opportunistic pathogenicity. It is crucial to conduct in vivo assays to validate these findings, given the potential risk to human health when used in biotechnological processes.

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