



# HARNESSING MULTIFUNCTIONAL PROBIOTIC YEASTS FROM DADIH A TRADITIONAL FERMENTED PRODUCT FROM WEST SUMATRA, INDONESIA

TATENDA CALVIN CHIHOMBORI



PROGRAM STUDY OF BIOTECHNOLOGY  
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IPB UNIVERSITY  
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Bogor, July 2024

Tatenda Calvin Chihombori  
P0501222801



## SUMMARY

TATENDA CALVIN CHIHOMBORI. Harnessing multifunctional probiotic yeasts from dadih a traditional fermented product from West Sumatra, Indonesia. Supervised by RIKA INDRI ASTUTI and APON ZAENAL MUSTOPA

Fermented buffalo milk, commonly called Dadih, contains potential probiotic yeast cells. Probiotic yeast has garnered increasing attention in basic and clinical science over the past 20 years. This research aimed to isolate, characterize, and determine the biodiversity of potential probiotic yeasts obtained from Dadih.

Isolation of probiotic yeast was done using Yeast Extract, Peptone, and Dextrose (YPD) media, followed by the identification of yeast using Sequence-Related Amplified Polymorphism (SRAP). Colonies relatedness was determined using the Numerical Taxonomy and Multivariate Analysis System (NTSYS). Two yeast isolates were further identified using Polymerase Chain Reaction (PCR) targeting the 18S rRNA gene. The probiotic characterization was done by determining pH resistance, bile salt resistance, proteolytic, lipolytic test, and hemolytic tests. Secondary metabolites were determined by conducting antioxidant, antidiabetic, antiinflammation, and antimicrobial tests. Interaction between secondary metabolites signals was determined by molecular docking using AutoDock software. Whole genome sequencing on *Pichia kudriavzevii* using Oxford Nanopore Technologies and genome annotation was performed using the Rapid Annotation using the Subsystem Technology (RAST) server. Fifty-five colonies were isolated, and their relatedness showed a minimum cutoff at a similarity level of 89% in the dendrogram. Two isolates, GBT 30 and GBT 37 were identified as *Pichia occidentalis* and *Pichia kudriavzevii*, respectively, based on the 18S rRNA sequence. Acid pH tolerance results showed both isolates survived at an acidic pH starting at pH 2 to pH 6. A bile salt resistance test showed that both isolates were able to show significant cell viability in 0.3 and 0.5% bile salt concentrations. Both isolates produced secondary metabolites, with GBT 30 showing good antimicrobial and antiinflammation properties than GBT 37. Whole-genome sequencing of *P. kudriavzevii* revealed a genome size of 10 906 850 bp, comprising five chromosomes with an average GC content of 38.26%, and secondary metabolites located on contig 7.

Keywords: *Pichia kudriavzevii*, *Pichia occidentalis*, dadih, fermentation, probiotic, whole genome sequencing

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## RINGKASAN

TATENDA CALVIN CHIHOMBORI. Memanfaatkan ragi probiotik multifungsi dari dadih, produk fermentasi tradisional dari Sumatera Barat, Indonesia. Dibimbing oleh RIKA INDRI ASTUTI dan APON ZAENAL MUSTOPA

Susu kerbau fermentasi yang biasa disebut Dadih mengandung sel ragi probiotik yang potensial. Ragi probiotik telah mendapatkan perhatian yang semakin besar dalam ilmu dasar dan klinis selama 20 tahun terakhir. Penelitian ini bertujuan untuk mengisolasi, mengkarakterisasi, dan mengetahui keanekaragaman hayati khamir probiotik potensial yang diperoleh dari Dadih.

Isolasi ragi probiotik dilakukan menggunakan media Yeast Extract, Peptone, dan Dextrose (YPD), dilanjutkan dengan identifikasi ragi menggunakan Sequence-Related Amplified Polymorphism (SRAP). Keterkaitan koloni ditentukan dengan menggunakan Numerical Taxonomy and Multivariate Analysis System (NTSYS). Dua isolat khamir diidentifikasi lebih lanjut menggunakan Polymerase Chain Reaction (PCR) dengan sasaran gen 18S rRNA. Karakterisasi probiotik dilakukan dengan menentukan ketahanan pH, ketahanan garam empedu, proteolitik, uji lipolitik, dan uji hemolitik. Metabolit sekunder ditentukan dengan melakukan uji antioksidan, antidiabetes, antiinflamasi, dan antimikroba. Interaksi antara sinyal metabolit sekunder ditentukan dengan molekuler docking menggunakan perangkat lunak AutoDock. Pengurutan seluruh genom pada *Pichia kudriavzevii* menggunakan Oxford Nanopore Technologies dan anotasi genom dilakukan menggunakan Rapid Annotation menggunakan server Subsystem Technology (RAST). Lima puluh lima koloni diisolasi, dan keterkaitannya menunjukkan batas minimum pada tingkat kemiripan 89% pada dendrogram. Dua isolat, GBT 30 dan GBT 37 masing-masing diidentifikasi sebagai *Pichia occidentalis* dan *Pichia kudriavzevii*, berdasarkan urutan 18S rRNA. Hasil toleransi pH asam menunjukkan kedua isolat mampu bertahan pada pH asam mulai pH 2 hingga pH 6. Uji ketahanan terhadap garam empedu menunjukkan bahwa kedua isolat mampu menunjukkan viabilitas sel yang signifikan pada konsentrasi garam empedu 0,3 dan 0,5%. Kedua isolat menghasilkan metabolit sekunder, dengan GBT 30 menunjukkan sifat antimikroba dan antiinflamasi yang lebih baik dibandingkan GBT 37. Pengurutan seluruh genom *P. kudriavzevii* menunjukkan ukuran genom 10.906.850 bp, terdiri dari lima kromosom dengan kandungan GC rata-rata 38,26%, dan metabolit sekunder yang terletak di contig 7.

Kata kunci: *Pichia kudriavzevii*, *Pichia occidentalis*, dadih, fermentasi probiotik, pengurutan genom utuh



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# **HARNESSING MULTIFUNCTIONAL PROBIOTIC YEASTS FROM DADIH A TRADITIONAL FERMENTED PRODUCT FROM WEST SUMATRA, INDONESIA**

**TATENDA CALVIN CHIHOMBORI**

Thesis  
as one of the requirements to obtain the  
Master degree on  
Biotechnology Study Program

**PROGRAM STUDY OF BIOTECHNOLOGY  
GRADUATE SCHOOL  
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External Examiner of Thesis Examination: Prof. Dr.Dra. Anja Meryandini M.S



Thesis Title: **Harnessing multifunctional probiotic yeasts from Dadih a traditional fermented product from West Sumatra, Indonesia**

Name : Tatenda Calvin Chihombori

NIM : P0501222801

Approved by

Main Supervisor:

Dr Rika Indri Astuti, M.Si

Co-Supervisor:

Dr Apon Zaenal Mustopa, M.Si

Agreed by

Head of Biotechnology Study Program:

Prof. Dr. Ir. Miftahudin, M.Si

NIP 196204191989031001

Dean for Postgraduate Studies:

Prof. Dr.Ir. Dodik Ridho Nurrochmat, M.Sc.F. Trop.,IPU

NIP 197003291996081001

Date of Thesis Examination:  
(05 July 2024)

Date of Graduation:

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The author hopes this thesis can be useful for readers, especially those interested in biotechnology. The author realizes that there are still many shortcomings in the preparation and writing of this thesis, so the author hopes for suggestions. Hopefully, this thesis can be useful for those who read it.

Bogor, July 2024

*Tatenda Calvin Chihombori*

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