

**ENZYMATIC AND MOLECULAR CHARACTERIZATION OF PHYTASE  
PRODUCING YEASTS ISOLATED FROM SOIL IN THE LIMPOPO  
PROVINCE**

**BY**

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## **DECLARATION**

I declare that the dissertation submitted to the University of Limpopo for the Masters of Science degree in Microbiology has not been submitted previously by me at this or other Institution, that it is my own work in design and that all materials contained in here has duly acknowledged.

Signed: \_\_\_\_\_

Date: \_\_\_\_\_

## **DEDICATIONS**

This work is dedicated to my family, my late Father and brothers Salani, Fhumulani and Ntshavheni Makhode, to my mom Alidzuli, my two brothers Lufuno and Mbengeni, my three sisters Tshiwela, Balanganani and Nkhumeleni Makhode, for their support, encouragement and love throughout my studies. God bless.

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## ABSTRACT

Twenty three phytase producing yeasts were isolated from soil in the Limpopo Province. The Limpopo Province has been found to be an ideal source of yeast able to function at high temperatures. The focus of this study was to profile the yeast isolates in terms of their phytase activities in order to confirm and establish the organism with the highest phytase activities. Three best phytase producing yeasts, HBD6.2, LD9 and LD7 were selected for further studies and illustrated activities of 676.02, 630.21 and 440.94 U.ml<sup>-1</sup>, respectively. HBD6.2, LD9 and LD7 were identified as *Candida guilliermondii*, *Candida diddensiae* and *Candida famata*, respectively, using standard conventional identification methods and PCR-RFLP was used to confirm the identities of these yeast isolates. Six known yeast strains obtained from the yeast culture collection (University of the Free State) were used as reference strains in the analysis. *Pichia guilliermondii* Y0209, *Pichia guilliermondii* Y0053 and *Pichia guilliermondii* Y0054 were used as reference strains for HBD6.2; *Candida diddensiae* for LD9; *Debaryomyces hansenii* Y0210 and *Debaryomyces hansenii* Y0610 as the reference strains for LD7. Eleven restriction digestion profiles used generated 84 markers with primers NS1 (5' GTAGTCATATGCTTGTCTC 3') and ITS2 (5' GCTGCGTTCTTCATCGATGC 3'). The similarity matrix was generated with the DICE coefficient using the NTSYS (pc) program. The genetic distance between the taxa was used to generate a UPGMA (unweighted pair group method using arithmetic averages) phylogenetic tree with a bootstrap of 100 replications using the Treecon program. The three test yeasts did not cluster with their reference strains, however, *C. guilliermondii* HBD6.2 clustered closely with *C. diddensiae* Y0774 at a bootstrap value of 90 and have a similarity level of 100 %. *C. diddensiae* LD9 and *C. diddensiae* Y0774 are both within the same cluster separated by a bootstrap of 65, but shared a genetic similarity of 87 %. *Candida famata* LD7 was found to be distantly related to all the yeast strains and it was only genetically similar to its reference strains *D. hansenii* Y0209 and *D. hansenii* Y0610 at 51 % and 48 %, respectively. To determine the optimal growth and enzyme activities, the three yeast isolates were grown in PSM broth in shake flasks at temperature ranges of 25, 30 and 35° C and the following pHs 4.0, 4.5, 5.0, 5.5 and 6.0, for each temperature. The optimum growth temperature of the three test yeasts was 30° C for HBD6.2 at pH 5.5, LD9 at pH

6.0, and LD7 at pH 5.5 or 6.0. The maximum enzyme activity was also obtained when the organisms were grown at 30° C. Maximum enzyme activity for HBD6.2 and LD9 was reached at pH 5.0 or 6.0, LD7 at pH 5.5 or 6.0. Phytases from all three yeast isolates were stable at 35° C, pH 5.5 for an average of 3 hrs retaining almost 80 % residual activity under these optimal conditions.

## LIST OF ABBREVIATIONS AND TERMINOLOGY USED

<i>AluI</i>	Restriction enzyme obtained from <i>Arthrobacter luteus</i>
BSA	Bovine serum albumin
Ca <sup>2+</sup>	Calcium
<i>CfoI</i>	Restriction enzyme obtained from <i>Clostridium farmicoaceticum</i>
DNA	Deoxyribose nucleic acid
dNTP	Deoxyribonucleoside triphosphate
<i>DraI</i>	Restriction enzyme obtained from <i>Drosophila spp.</i>
DTT	Dithiothritol
EDTA	Ethylenediaminetetraaceticacid
<i>HaeIII</i>	Restriction enzyme obtained from <i>Haemophilus aegyptius</i>
HAPs	Histidine acid phosphatases
<i>HpaII</i>	Restriction enzyme obtained from <i>Haemophilus parainfluenzae</i>
Ins	Inositol
IP <sub>6</sub>	Inositol hexaphosphate
ITS	Internal transcribed spacer
K <sup>+</sup>	Potassium
Mg <sup>2+</sup>	Magnesium
<i>MspI</i>	Restriction enzyme obtained from <i>Moraxella spp.</i>
P	Phosphorus
PAGE	Polyacrylamide gel electrophoresis
PAP	Purple acid phosphatases
PCR	Polymorphic chain reaction
Pi	Inorganic phosphorus
PMSF	Phenyl methanesulfonyl fluoride
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
<i>RsaI</i>	Restriction enzyme obtained from <i>Rhodopseudomonas sphaeroid</i>
SDS	Sodium dodecyl sulfate
SmF	Submerged fermentation

SSF	Solid state fermentation
<i>TaqI</i>	Restriction enzyme obtained from <i>Thermas aquaticus</i>
TCA	Trichloroacetic acid
TE buffer	Tri, 2-ethylene-diamine-tetra-acetate buffer
TEMED	Tetramethyl-ethlenediamine
Zn <sup>2+</sup>	Zinc
βPP	β-propeller phytase



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