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⁻¹, 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′ GCTGCCTTCTTCATCGATGC 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 35º 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 30º 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 30º 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 35º 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

*AluI*  Restriction enzyme obtained from *Arthrobacter luteus*
BSA  Bovine serum albumin
*Ca$^{2+}$*  Calcium
*CfoI*  Restriction enzyme obtained from *Clostridium farmicoaceticum*
DNA  Deoxyribose nucleic acid
dNTP  Deoxyribonucleoside triphosphate
*DraI*  Restriction enzyme obtained from *Drosophila spp.*
DTT  Dithiothritol
EDTA  Ethylenediaminetetraaceticacid
*HaeIII*  Restriction enzyme obtained from *Haemophilus aegyptius*
HAPs  Histidine acid phosphatases
*HpaII*  Restriction enzyme obtained from *Haemophilus parainfluenzae*
Ins  Inositol
*IP$_6$*  Inositol hexaphosphate
ITS  Internal transcribed spacer
*K$^+$*  Potassium
*Mg$^{2+}$*  Magnesium
*MspI*  Restriction enzyme obtained from *Moraxella spp.*
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
*RsaI*  Restriction enzyme obtained from *Rhodopseudomonas sphaeroid*
SDS  Sodium dodecyl sulfate
SmF  Submerged fermentation
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<tr>
<td>SSF</td>
<td>Solid state fermentation</td>
</tr>
<tr>
<td>TaqI</td>
<td>Restriction enzyme obtained from <em>Thermus aquaticus</em></td>
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<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>TE buffer</td>
<td>Tri, 2-ethylene-diamine-tetra-acetate buffer</td>
</tr>
<tr>
<td>TEMED</td>
<td>Tetramethyl-ethylenediamine</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>Zinc</td>
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<td>βPP</td>
<td>β-propeller phytase</td>
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