Development of PCR primers for the detection of Family VII lipolytic genes in bacterial and environmental genetic materials

Tlou MG* and Nthangeni MB

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, P. O. BOX 339, BLOEMFONTEIN, 9300, SOUTH AFRICA

Lipolytic enzymes are in terms of potential applications, the most versatile enzymes. Bacterial lipolytic enzymes have been classified into eight families based on conserved amino acid sequence motifs and biological properties. The alignment of selected protein sequences encoding carboxylesterases that belong to Family VII bacterial lipolytic enzymes enabled us to identify four blocks of relatively conserved amino acid sequences that we used to design degenerate primers for the detection of the presence of Family VII lipolytic genes in genetic materials. The PCR using degenerate primers based on Block 1 and Block 3 (respectively corresponding to amino acid residues 24-35 and 175 -191 of pnitrobenzyl esterase from *Bacillus subtilis*) detected the presence of carboxylesterase genes in all the test strains and were subsequently used to detect the presence of Family VII bacterial lipolytic genes in genetic materials isolated from different environmental soil samples. The expected PCR products of about 500 bp were obtained and subjected to RFLP to identify the heterogeneity of the amplified DNA fragments. The representative of each RFLP pattern was selected for sequencing. Nucleotide and protein sequence analysis revealed the cloning of diverse DNA fragments encoding novel members of Family VII carboxylesterases.