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Heterologous expressionof lipase YLIP9 from *Yarrowia lipolytica* MSR80: Effect of host-vector combinations, fusion tags and codon usage

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A gene encoding a thermostable, methanol-stable and enantioselective lipase YLIP9 of *Yarrowia lipolytica* MSR80 was cloned and expressed in pEZZ10-HB101 vector-host system. pEZZ18 vector has a 14 kDa ZZ-tag and the purified protein of 60 kDa was obtained. In this system, constitutive extracellular expression of ZZ-YLIP9 was observed, after 48 hours incubation at 37° C/300 rpm with expression level of 0.25±0.15 U/ml. As the titres were low, this gene was sub-cloned and expressed in vectors with different tags-pET22b (C-terminal His-tag), pET51b (N-terminal Strep and C-terminal His-tag), pET22b-SUMO (N-terminal His, 10 kDa SUMO-tag and C-terminal His-tag) and pGEX-4t1 (N-terminal 26 kDa GST-tag) with protein of 45, 45, 55 and 70 kDa, respectively. Periplasmic expression in pET22b and pET22b-SUMO and intracellular expression in pET51b and pGEX-4t1 was observed 3 h after IPTG induction. In pET22b the expressed protein was inactive while expression of 3.25±0.13, 3.5±0.09 and 1.75±0.21 U/ml was obtained with pET51b, pET22b-SUMO and pGEX-4t1, respectively. Around 14-fold enhancement was observed with pET22b-SUMO. However, the expression was not substantial so the usage of codons in *E. coli* was compared to that of *Y. lipolytica* using the codon usage database. About 80% of the codons were changed and the gene was synthesized, re-sequenced followed by its sub cloning and expression in pET22b, pET51b, pET22b-SUMO and pEZZ18 vector-*E. coli* host systems. As compared to earlier observation this time the protein that was expressed in pET22b was active with an expression level of 2.98±0.18 U/ml and a further 2-3 fold enhancement was observed for pET51b and pET22b-SUMO, whereas, a significant 40-fold enhancement was observed in pEZZ18-HB101 system. Hence, host-vector combinations, tags and codon usage have a significant effect on protein expression.

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Expression analysis of novel small RNAs of *Propionibacterium acnes* KPA171202

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Propionibacterium acnes is an anaerobic, gram-positive, opportunistic pathogen known to involved in wide variety of diseases ranging from mild acne to prostate cancer. Bacterial small RNAs vary in length from approximately 50 to 600 nucleotides and are usually not translated into proteins. They are encoded from the intergenic regions and are only expressed under highly specific growth conditions. They are recognized as novel regulators of gene expression and are known to be involved in virulence, pathogenesis, stress tolerance & adaptation to environmental changes in bacteria. Present study was undertaken keeping in view the lack of predicted sRNAs of *Propionibacterium acnes* KPA171202 in databases. This report represents first attempt to identify sRNAs in P. acnes. A total of eight potential candidate sRNAs were predicted using SIPHT, one was found to have Rfam homolog and seven were novel. Out of these seven predicted sRNAs, five were validated by reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing. The expression of these sRNAs was quantified in different growth phases by qPCR (Quantitative PCR). Also, the expression of one of the sRNA: sPPAK4 was analyzed under heat shock. They were found to be expressed in all stages of growth but with varied expression level which points to regulatory role for them. Further investigation of their targets and regulatory functions is in progress.

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