

of ellipticity for the mesostable (Est95 and Est96) *versus* the thermostable enzyme (Est179), a possible indication of an increased number α -helices in Est179. This is further supported by previous studies that have suggested that most thermostable proteins have high contents of α -helix [30, 31, and 32].

On the other hand, the high intrinsic fluorescence intensity exhibited by Est179 indicates that Trp residues are more exposed to solvent in this thermostable CEST when compared to the mesostable counterparts. This was in agreement with the fluorescence spectrum of thermophilic esterase reported by [21]. According to [33], Trp residues may contribute to structural stability by their hydrophobicity and π -cation interactions. It is noteworthy that the 3D structure of *G. stearothermophilus* CEST, a member of Family VII lipolytic proteins is available [10]. This could facilitate protein modeling to deduce the structural basis for enhanced thermostability which is one preferred prerequisite for CEST implementation in biocatalytic processes.

V. CONCLUSION

CEST from mesophilic *B. pumilus* and *B. licheniformis* species and a putative CEST from *G. kaustophilus* HTA426 were successfully cloned, over-expressed in *E. coli* and purified to near homogeneity in one step purification using nickel affinity chromatography technique. The study demonstrated the significance of gene codon adaptation which enabled successful expression of the *G. kaustophilus* CEST gene in *Escherichia coli*. The adoption of gene optimization strategies could greatly facilitate the expression of genes discovered during genome and metagenome sequences that would otherwise fail to express in heterologous hosts. The CESTs were active toward short-length p-NP esters, with high stability and activity under alkaline pH. The CEST from *G. kaustophilus* is a good candidate for application in biotechnology industry given its thermostability and activity in alkaline pH. The *Geobacillus stearothermophilus* CEST is available as template for three-dimensional structure modelling to elucidate structure-function properties of Family VII lipolytic proteins allowing rational protein design and tailoring the enzyme for a given application.

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