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A Review on Molecular Structural Characterization of Human Cysteine Cathepsins in *Escherichia coli* Expression Systems

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Background: Eleven cysteine cathepsins have been identified in human, Cathepsin B, C, F, H, K, L, O, S, V, X and W. Studies related to their specific functions, regulation and distribution patterns in tissues have not been fully studied to understand their biochemical implications in human physiology. Molecular characterization including expression and recombinant production of them in bacterial expression systems is an effective way of understanding them.

Objectives: To identify research gaps present in molecular structural characterization studies of human cysteine cathepsins highlighting the importance of investigating them to promote health.

Methodology: This review focused on molecular structural characterization studies that have been done so far based on *in vitro* expression of genes encoding for human cysteine cathepsins in *Escherichia coli* expression systems. Nearly 50 related papers were found as published literature using keywords cysteine cathepsins, expression and *E. coli* in global databases such as the Google Scholar, PUBMED and NCBI and were analysed.

Results: It was seen that all cathepsins except for K, C, H, X and W have been expressed in bacterial expression systems, the majority in *E. coli BL21(DE3) pLysS* host via *pET3* expression vector to understand cellular behaviour. In most cases, the substrate used to validate the enzymatic activity of the recombinant enzyme was a cysteine residue along with a *benzyloxy-carbonyl* salt such as *benzyloxycarbonyl-L-phenylalanyl-L-arginine-7-amido-4-rnethylcoumarln.* No literature indicated that cathepsins K, C, H, X and W to have been characterized on any molecular basis.

Conclusions: It is concluded that certain important research gaps such as precise validation of the recombinant cysteine cathepsin produced needs to be attended to by investigating into specific substrates utilized by each enzyme. In addition, mass production of these enzymes have to be facilitated by optimizing their recombinant production efficiency, in order for them to be incorporated into biopharmaceuticals productively.

Keywords: *Cathepsins, Escherichia coli, Expression, Molecular, Characterization*

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