PRODUCTION OF UNSULFATED CHONDROITIN AND ASSOCIATED CHONDRO-OLIGOSACCHARIDES IN RECOMBINANT *ESCHERICHIA COLI*

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Glycosaminoglycans (GAGs) are key components of vertebrate extracellular matrices. Among them, chondroitins are distinguished by a polysaccharide backbone composed of repeating disaccharide units of β4-glucuronic acid (β4GlcUA) and β3-N-acetylgalactosamine (β3GalNAc). Chondroitin is typically sulfated, which defines the family of chondroitin sulfates, although unsulfated forms of chondroitin can be found in certain bacteria [1]. Given their involvement in various biological processes, chondroitin oligosaccharides are promising molecules for chemical or enzymatic modifications, such as sulfation, to yield well-defined biologically active compounds [2]. The bacterial production of oligosaccharides presents a viable alternative to in vitro synthesis, as it allows for the integrated synthesis of all polysaccharide substrates, nucleotide sugars, and enzymes in the recombinant host, effectively functioning as a living enzymatic reactor. This method has already been successfully applied to produce heparosan oligosaccharides [3] and, more recently, di- and tetrasaccharides of chondroitin [4]. In this study, we engineered non-pathogenic strains of *Escherichia coli* to produce unsulfated chondroitin, both with and without chondroitin lyase, to generate either the chondroitin polymer or its associated oligosaccharides. Chondroitin was synthesized using chondroitin synthase KfoC from *E. coli K4*, and degradation of chondroitin was achieved with chondroitin lyase from *Victivallis vadensis* ATCC BAA-548 [5], acting as a true endo-enzyme to produce a broad range of oligosaccharides, from trimers to 18-mers. This is the first report of the microbial production of large chondro-oligosaccharides.

References:

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