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Use of white biotechnology for the production of dimerized human bone morphogenetic protein 2 in Bacillus subtilis

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Bone Morphogenetic Protein 2 (BMP2) has an important clinical application in the spine fusion and ortho/maxillofacial surgeries. Here we first report the secretory expression of recombinant human BMP2 from *Bacillus substilis* system. The mature domain of BMP2 gene (Accession no. KF250425) was amplified from pTz57R/BMP2 plasmid. Two constructs were designed; one with single BMP2 gene and the other having two mature BMP2 genes coupled with glycine serine rich linker to produce a dimer. Both the constructs were cloned into the pHT43 expression vector and sequence analyzed. For secretory expression analysis and optimization, the pHT43-BMP2-M and pHT43-BMP2-D vectors were transformed into two different strains of *Bacillus subtilis* i.e. SCK6 and WB600 respectively. Expression conditions like media and temperature were optimized and the maximum 35% and 25% secretory expression of monomer (~13kDa) and dimer (~25kDa) respectively was achieved in 2xYT medium at 30°C in SCK6 strain. The expression and dimeric nature of the BMP2 was confirmed by western blot and Native PAGE analysis. For the purification of recombinant protein, 200ml culture supernatant was freeze dried to 10ml, dialyzed against the Tris-Cl (pH 8.5) and Fast Protein Liquid Chromatography, Resource Q (6ml) column was run. The recombinant human BMP2 monomer and dimer were eluted at 0.9M and 0.6M NaCl respectively. The biological activity of both the monomer and dimer BMP2 (0, 50, 100, 200 and 400 ng/ml) and commercially available positive control were analyzed by alkaline phosphatase (ALP) assay on C2C12 cells. The results showed maximum ALP activity at 200ng/ml in a dose dependent manner. In conclusion, our results showed the recombinant production of biological active dimerized BMP2 from Bacillus subtilis in culture media which was not previously reported

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