The Production of Hyaluronan by Recombinant Escherichia coli

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ABSTRACT

Hyaluronic acid is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine. Because of it ’ s special biocompatibility, moister-holding function and lubricant properties, it has been used as biomedical, cosmetic industry and other relevance fields. Traditionaly, hyaluronic acid is produced mainly from animal tissue extract. But this way of production is expensive and gives low yield, therefore is of no commercial use. Nowdays, the production of hyaluronic acid by microorganism of type A and type C streptococci is commercial available. The wild type streptococci produces aboundant hyaluronic acid to form built capsule, it also produces hemolycin and pathogenic toxins to extracellular environment. The application to industrialized production will select those mutants lacking toxic and pathogenic ability. But the mutated bacteria usually greatly reduce the ability to produce hyaluronic acid. The hyaluronic acid in streptococci is known to be produced by a synthatase controlled by an operon, which bears three genes. Consequently, a stradegy for a higher safety and long-term production of hyaluronic acid by the recombinant synthatase in E.coli DH5? is reported. The has operon, from S. pyogenes, and the constitutive expression promoter, ace, are constructed into E.coli, for used in hyaluronic acid production. The capsule in streptococci was not observed in one of this strain pACE-spHasABC. The differences in constructed strains, medium components, and treatments were explored the impact of the hyaluronic acid production. The experimental results showed that 27.22 mg / L of the glucuronic acid, one of the precursor of hyaluronic acid, were generated by the strain pACE-spHasBC, whereas 17.74 mg / L glucuronic acid, by the strain pACE-spHasABC. On the other side, with or without addition of the cofactor, MgCl2 and substrate glucosamine in the media, for the production of hyaluronic acid made insignificant difference. The hyaluronic acid production from the strains either containing shorter hyaluronic acid fragments in the transformation mixtures or co-transfer a plasmid that encode active part of hyaluronic acid synthase were also evaluated. The yield of hyaluronic acid from both strains is 30% higher than that of pACE-spHasABC alone. These results imply that the use of E.coli to produce hyaluronic acid need small fragments of hyaluronate as primer, and that has operon is also needed proteins for transferring the synthesized hyaluronate out of the cells.

Keywords : hyaluronic acid ; Streptococcus pyogenes ; operon ; constitutive promoter

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