Studies on Directed Evolution of Novel Transglycosidases

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The commercial interest in functional foods with medicinal properties that promote health beyond nutrition has recently increased dramatically all over the world because of the consumer's awareness of the link between health and nutrition. Galacto-oligosaccharides have proven ability to promote the growth of beneficial intestinal bacteria including Bifidobacteria. The commercial production of oligosaccharides is through the intervention of transgalactosylation property of glycosidases from several sources such as bacteria and fungi. Directed molecular evolution techniques represented by **DNA shuffling / Family shuffling** are the emerging fields of protein engineering which can be applied to enhance the **transgalactosylation activity** of **glycosidases.** In this study, we made an attempt to enhance the transgalactosylation function of β - glycosidases. Initially, the library was prepared in *E. coli* BL-21 (DE3), XL-1 Blue and Novablue (DE3) by subcloning the shuffled β - glucosidase using 50 bp fragments following Stemmer's method into pET-23d (+). The library was screened on LB agar plates containing ampicillin and a chromogenic substrate X - gal for β - galactosidase activity. However, the transformants obtained did not exhibit any β - galactosidase activity. Sequencing twelve of the randomly picked transformants revealed the presence of several stop codons that had been generated by a number of mutations and had prevented the expression of enzyme activity. As the mutation rate was high due to shuffling of small sized fragments, shuffling was then done using 200 bp fragments. The shuffled library was prepared both in *E. coli* XL-1 Blue and Novablue (DE3). The library prepared using shuffling of 200 bp fragments of both the genes in *E. coli* XL-1 blue / Novablue (DE3) gave a large number of transformants exhibiting β - galactosidase activity. On screening using X-gal, a total of 57 blue colonies out of 6,000 colonies of *E. coli* XL-1 blue and 356 blue colonies out of 17,000 colonies of Novablue (DE3) were picked up and subjected to second screening for measurement of enzyme activity using glucose oxidase assay. The two of the transformants namely B-51 and D-4 exhibited two to five times higher level of β - galactosidase activity as compared to the parent. However, the activity was located mainly in cell pellet. HPLC analysis also revealed the presence of higher quantity of transfer products as compared to the parent. The attempts to prepare library for second round of shuffling using the parent and other four transformants exhibiting higher β - galactosidase activity (selected form first library) using another vector pCorrectClone (pCC) / host are in progress.