

Effect of Size, ori Type, and cer DNA Fragment on Stability of Plasmid Carrying sod Gene

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Abstract

Problems that frequently occur in the production of the recombinant proteins are decrease stability of plasmid segregation and structure, resulting decrease protein production. Stability of plasmid segregation and structure affected by its structure, copy number and size of plasmid. This study aims to evaluate the effect of size, ori type and cer fragment on stability of plasmid segregation and structure, sodgene expression, and rSOD activity. These plasmids are pJexpress_sod, pBM_sod, pCAD_sod, and pCAD_sod_dapD_cer. Stability of plasmid segregation was analysed using replica plating, agarose gel electrophoresis for stability of plasmid structure, sod gen expression used by SDS-PAGE and rSOD activity used by zymography with natif-PAGE. The stability of the pCAF_sod_dapD_cer segregation is the best and pJexpress_sod is the most unstable. Cer fragment inserted on pCAD_sod_dapD_cer was able to improve the stability of segregation, in addition, it can increase the stability of plasmid segregation by as much as 1,7-1,8 times than pCAD_sod. The stability of plasmid size is not affected by stability of plasmid segregation. rSOD protein size is stable when the stability of plasmid segregation less than 90%. The intensity of the rSOD protein bands from plasmid pCAD_sod_dapD_cer is the smallest and pBM_sod is the greatest when the stability of segregation less than 90%. In addition, the enzymatic activity of rSOD decreased when the stability of plasmid segregation is less than 90%. rSOD from pCAD_sod is the most decreases about enzymatic activity. The results show those size, ori type, and cer DNA fragment affect the stability of segregation, the intensity of the protein resulted, and rSOD activity, but not for the stability of the size on the pJexpress_sod, pBM_sod, pCAD_sod dan pCAD_sod_dapD_cer plasmids.

Key words : size plasmid, ori type, cer fragment, stability of segregation and structural plasmid