Abstract

In bioethanol production, the enzymatic saccharification of lignocellulosic biomass for release of reducing sugars is the cost limiting step. Therefore, for reducing the production cost of bioethanol, strain improvement of cellulase producing microorganisms is important. Random mutagenesis by UV in microorganism resembles the natural evolution process. Therefore, it can be used for improvement of biochemical properties in industrial enzymes (cellulase). Wild-type strain of *Bacillus amyloliquefaciens* SS35 was exposed to UV irradiations to develop the UV2 mutant strain with improved endoglucanase catalytic efficiency and wide range pH stability. The gene encoding endoglucanase, *Ba*GH5-WT and *Ba*GH5-UV2 were amplified from wild-type and UV2 mutant of *Bacillus amyloliquefaciens* SS35, respectively, using degenerate primers for family 5 glycoside hydrolase (GH5) and were cloned in pET-28a(+) vector and expressed in *E. coli* BL21(DE3) pLysS cells. The recombinant mutant *Ba*GH5-UV2 showed 22-fold higher catalytic efficiency and wider range pH stability than recombinant wild-type *Ba*GH5-WT. The mutant enzyme, *Ba*GH5-UV2 showed substitution mutation of residue, Asp256 to Gly256. This mutation was in loop connecting the β_6 to α_6 of $(\beta/\alpha)_8$ TIM-barrel fold. Molecular dynamics simulation studies showed more stable 3-D structure for *Ba*GH5-UV2 than *Ba*GH5-WT. Molecular docking results showed that *Ba*GH5-UV2 gave maximum increase in Gibb's free energy ($\Delta G°$) against cellotetraose. Application of *Ba*GH5-UV2 in saccharification of acid or base pretreated *Sorghum durra* stalk in cocktail with *Ct*CBH5A and *Ct*GH1 was explored for pretreatment specific customization of enzymes in mixture design. This report provides the information for protein engineering in GH5 endoglucanases for improving their biochemical properties and pretreatment specific optimization of enzymes in mixture for enzymatic saccharification.