Title:	Bioinformatics analysis and cloning of potent thermostable endoglucanases for biomass degradation and Structural characterisation and activity assay of engineered Pyrococcus furiosus DNA ligases
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Abstract:	Efficient enzymatic depolymerisation is an essential step in the process of production of 2nd generation bio fuels from biomass. Cellulose being the most abundant constituent of biomass is a regenerative source of biomass energy but is robust and recalcitrant in nature. Thermostable cellulases and enzymes from biomass degrading cellular machinery, cellulosome can prove to be good candidates for use of cellulose deconstruction. Here, we present a list of potent thermostable endoglucanases shortlisted by comprehensive bioinformatics analysis using homology modelling. Further, a similar analysis was carried out to enlist potent cellulases from the thermostable anaerobe, Clostridium thermocellum. We also describe the attempts in cloning some of the potent thermostable cellulases from Clostridium thermocellum genome. DNA ligase is an indispensably important enzyme that seals nick in DNA backbone. In molecular biology experiments, T4 DNA ligase has been used commercially. The limitation of T4 DNA ligase exists in its thermostability and in dealing with the ligation of the DNA template forming a secondary structure at low temperature. In order to overcome these shortcomings, two mesoactive thermostable DNA ligases were engineered from Pyrococcus furiosus WT DNA ligase. Here, we describe the expression and purification of these two engineered ligases, namely ΔPfu ligase and PfuT4H ligase. Through structural characterisation, both novel ligases were observed to be extremely thermostable as well as resistant to chemical denaturation by urea. Both of the ligases were partly denatured by high concentration of guanidium hydrochloride. On comparison of enzymatic activity with T4 DNA ligase, activity was observed to be more for both ligases at room temperature.
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