Enhancement of Stability and Catalytic Activity of Xylanase A under Alkaline Conditions by Directed Evolution

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The activity and stability of xylanases from *Bacillus* sp. were improved by directed evolution. Two mutants (D3 and D9) were selected by sequential rounds of error-prone PCR to introduce random mutations and membrane-based screening of the resultant mutant library. Both D3 and D9 had four amino acid substitutions. Two mutants (M32 and M32D9) was constructed by site-directed mutagenesis. They had six and seven amino acid substitutions respectively. All the substituted amino acids were located on the surface of protein and some substituted amino acids had non-polar residues with hydroxyl group.

The activity and stability of four mutants (D3, D9, M32, and M32D9) were enhanced in alkaline conditions, compared to that of wild type. Especially M32D9 had 4-fold higher enzyme activity than the wild-type enzyme at pH 9.0. It also displayed increased alkalostability. It maintained about 89% of its initial activity even after incubation for 18 days, whereas the wild-type enzyme lost 50% of its activity after 6 days at pH 9.0. In addition to alkalostability, thermostability was also improved in D3, D9, and M32D9.

Mutant xylanase (M161) was expressed using recombinant *Bacillus subtilis* harboring pSMX-M161 vector. In semi-defined medium containing 20 g/l of glucose and 3 g/l ammonium sulfate, 11.7 U/ml and 38 U/ml of xylanase activity were obtained in 18 hours of batch cultivation and 22 hours of fed batch cultivation respectively.