Directed evolution of xylanase J from alkaliphilic *Bacillus* sp. strain 41M-1: restore of alkaliphily of a mutant with an acidic pH optimum

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**ABSTRACT**

Alkaliphilic *Bacillus* sp. strain 41M-1 produces an alkaliphilic xylanase (xylanase J). The newly constructed mutant E177QAJC had an acidic pH optimum and showed almost no activity at pH 8.0. The alkaliphily of the enzyme was restored by directed evolution. The evolved mutants, Y176S/E177QAJC and G32V/Y176D/E177QAJC, retained about 30% and 43% activity of their maximal activities at pH 6.0, respectively.

**INTRODUCTION**

Xylan, the major component of hemicellulose, has a backbone of β-1,4-linked xylopyranosyl residues. Xylanase catalyzes the hydrolysis of xylan to xylo-oligosaccharides and xylose. Since solubility of xylan in aqueous solution increases at alkaline pHs, xylanases work under alkaline conditions are required, especially for industrial applications.

Xylanase J (XynJ) from alkaliphilic *Bacillus* sp. strain 41M-1 has an alkaline pH optimum and is composed of a family 11 catalytic domain and a xylan-binding domain (XBD) [1]. Many XynJ mutants with acidic and neutral pH optima have previously been obtained by a single amino acid substitution in the catalytic domain [2]. Of all the mutants, mutant E177Q (Glu177 is substituted by Gln) is the most acidophilic one with a pH optimum of 5.0.

In this study, we shifted the pH optimum of mutant E177Q to the alkaline side as a first step of creating more alkaline-active XynJ mutants by the suppressor mutation procedure [3].

**EXPERIMENTAL AND DISCUSSION**

The DNA fragment encoding the catalytic domain region of mutant E177Q was amplified from expression plasmid pAXT3R-E177Q [2] using primers which allow directional inframe ligation to expression vector pET-21b(+). The constructed expression plasmid pET-E177QAJC was transformed into *Escherichia coli* BL21(DE3). The recombinant enzyme E177QAJC lacking the XBD had an acidic pH optimum just like E177Q composed of the catalytic domain and XBD.

Random mutations were introduced to the catalytic domain region of mutant E177Q gene of pET-E177QAJC by error-prone PCR. The PCR products were ligated into pET-21b(+) and introduced to *E. coli* BL21(DE3) to construct a mutation library. Evolved mutants were screened for enhanced activity at alkaline pHs by the filter
assay. In this method, xylanase activity at alkaline conditions is detected as a halo-forming ability by transferring E. coli transformants grown on a nitrocellulose filter to a xylan-containing alkaline plate. Two clones with restored alkaliphily, Y176S/E177QAJC and G32V/Y176D/E177QAJC were selected out of 7,000 clones. They contained Y176S/E177Q and G32V/Y176D/E177Q amino acid substitutions, respectively. As shown in Figure 1, mutant E177QAJC exhibited the highest activity at pH 5.0 and has almost no activity at pH 8.0 (panel A), while the optimum pHs of the evolved mutants Y176S/E177QAJC and G32V/Y176D/E177QAJC were both pH 6.0 and they retained about 30% and 43% activity at pH 8.0 of their maximal activities, respectively (panels B and C).

Currently, we are investigating the influences of mutations G32V, Y176 and Y176S on wild-type XynJ.

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REFERENCES

Figure 1. Effect of reaction pH on activity of mutants E177QAJC (A), Y176S/E177QAJC (B) and G32V/Y176D/E177QAJC (C). An activity of a cell free extract of each transformant was measured at 37°C. ●: citrate buffer (pH 4.0-6.0), ▲: phosphate buffer (pH 6.0-8.0), ◆: Tris buffer (pH 8.0-9.0), ×: carbonate buffer (pH 9.0-11.0).