APPENDIX 3

SEQUENCE DETAILS OF GS57308 pUC19-XYN2 SYSTEM

Table A.2 Sequence details of GS57308 pUC19-Xyn2 system

<table>
<thead>
<tr>
<th>LOCUS</th>
<th>GS57308 pUC19-xyn2</th>
<th>3534 bp</th>
<th>DNA</th>
<th>circular SYN</th>
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<tr>
<td>31-MAR-2015</td>
<td></td>
<td></td>
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</tr>
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</table>

DEFINITION Ligation of inverted xyn2 into pUC19

ACCESSION GS57308 pUC19-xyn2

KEYWORDS .

SOURCE Unknown.

ORGANISM Unknown

Unclassified.

REFERENCE 1 (bases 1 to 3534)

AUTHORS Self

JOURNAL Unpublished.

COMMENT SECID/File created by Clone Manager, Scientific & Educational Software

COMMENT SECNOTES|Vector molecule: pUC19

Fragment ends: SmaI

Fragment size: 2686

Insert molecule: xyn2

Fragment ends: blunt

Fragment size: 840

FEATURES misc_feature complement(250..398) /gene="lacZ"
A number of xylanases have been isolated from various acidophilic and alkaliphilic microorganisms ever since their initial finding from Bacillus sp. C-59-2 (Horikoshi & Atsukawa 1973). These include family 10 and 11 xylanases from a number of Acidobacterium sp. (Inagaki et al 1998), Aspergillus sp. (Fushinobu et al 1998; Krengel & Dijkstra 1996), Penicillium sp. (Kimura et al 2000), Bacillus sp. (Sabini et al 2001; Khasin et al 1993; Nakamura et al 1993), Cryptococcus sp. (Iefuji et al 1996) and Trichoderma sp. (Torronen & Rouvinen 1995; Torronen et al 1994; Torronen et al 1992) in addition to family 8 xylanases from alkalophilic B. halodurans C-125 (Takami et al 2000; Takami & Horikoshi 2000) and B. halodurans MIR32. The amino acid sequence analysis pointed out that these could be intracellular enzymes and thus might not be modified to the environment of their hosts. Many of the alkaliphilic microorganisms were found to produce xylanases with pH optima in the near neutral region but with relatively high activities being reserved in alkaline conditions. A number of xylanases with more alkaline pH optima have also been isolated and one the most alkaliphilic xylanases is XylB from Bacillus sp. AR-009 that has a pH optimum of pH 9 to 10 (Gessesse 1998). Other highly alkaliphilic xylanases include xylanase J from Bacillus sp. strain 41M-1 (Christakopoulos et al 1996) and a xylanase from Bacillus pumilus 13a (Duarte et al 2000) having an optimum pH of 9. The most important acidophilic xylanases are the family 10 and 11 members from Aspergillus kawachii (Fushinobu et al 1998; Ito et al 1992), A. niger (Krengel & Dijkstra 1996), Cryptococcus sp. S-2 (Iefuji et al 1996) Penicillium sp. 40 (Kimura et al 2000) and T. reesei (Torronen & Rouvinen 1995). The most acidophilic of these have a pH optimum of 2 and stability over a broad pH range.
and unfolding (Collins et al. 2003). For instance, pXyl has a 12 times shorter half life of inactivation than a mesophilic xylanase at 55 °C and also pXyl shows a 10°C decrease in melting temperature compared to mesophilic reference xylanases. Fluorescence monitoring of acrylamide quenching indicated that the family 8 cold adapted xylanase has an amplified flexibility compared to a thermophilic homologous enzyme like CelA from *C. thermocellum*, other than a increased low temperature activity and reduced stability (Collins et al. 2003).

When compared to a thermophilic and mesophilic homolog, the family 8 and yeast cold adapted xylanases are both distinguished by a number of discrete modifications that might give rise to a decrease in the stability, and hence an increase in the molecular structure flexibility. While the family 10 yeast xylanase is characterized by a less compact hydrophobic packing with the loss of one salt bridge and a destabilization of the helix macrodipoles (Petrescu et al. 2000), the *Pseudoalteromonas haloplanktis* TAH3 α xylanase is characterised by a reduced number of salt bridges and an increased exposure of hydrophobic residues (Van-Petegem et al. 2002; Van-Petegem et al. 2003). These modifications are an extension of those observed between thermophilic and mesophilic xylanases. Auxiliary analysis of psychrophilic xylanases, particularly relative studies with more closely related and better characterised homologs are still essential for better understanding of the temperature adaptation in these enzymes.

1.2.3.1.3 Alkaliphiles and acidophiles

Habitats with extreme pH are also common, especially in carbonate laden soils, geothermal regions, soda deserts and lakes, despite the fact that the majority of natural environments on earth are essentially neutral with pH values between 5 and 9. Undeniably xylanase producing alkaliphilic microorganisms and acidophiles have been isolated from these environments (Kimura et al. 2000; Horikoshi 1999; Gessesse 1998) and also from sources like decomposing organic matter (Duarte et al. 2000), faeces (Horikoshi 1999), kraft pulp (Yang et al. 1995), etc.
Van-Petegem et al 2002; Van-Petegem et al 2003) and Flavobacterium frigidarium sp. nov. (Humphry et al 2001), a gram positive bacterium named Clostridium strain PXYL1 (Akila & Chandra 2003), a yeast isolate named Cryptococcus adeliae (Petrescu et al 2000), krill called Euphasia superba (Turkiewiz et al 2000), a number of fungi like Penicillium sp., Alternaria alternata and Phoma sp. 2 (Bradner et al 1999) and a number of basidiomycetes like Coprinus psychromorbidus (Inglis et al 2000) are found to produce psychrophilic xylanase. Though all these have been isolated from the Antarctic environment, studies of the xylanases produced from sources other than the Pseudoalteromonas haloplanktis TAH3 α(pXyl) and the Cryptococcus adeliae are minimal. High catalytic activities at low temperatures, poor stability and a low temperature optimum are the common features of the psychrophilic xylanases (Collins et al 2002; Feller & Gerday 2003; Georlette et al 2004). Relative studies of pXyl with mesophilic xylanases demonstrated that these enzymes have a higher catalytic activity at low and moderate temperatures having 10 times higher activity at 5 °C and 3 times higher activity at 30 °C (Collins et al 2002). Besides, all psychrophilic enzymes displayed high catalytic activity at low temperatures. Activity of pXyl is 60% of the maximum whereas xylanases A and B from Euphasia superba exhibited approximately 30% and 40% of their maximum activity respectively at 5 °C. A mesophilic xylanase in contrast, showed less than 5% of its maximum activity at this temperature (Collins et al 2002). Similarly, the apparent optimal temperatures for activity of pXyl and the microfungal xylanases are approximately 25 °C and 10 to 30 °C lower than that of the mesophilic reference xylanases used providing further evidence for the adaptation of these enzymes to cold environments. Poor thermal stability of the psychrophilic xylanases was indicated by short half lives and low denaturation temperatures whereas a lower chemical stability of the cold adapted family 8 xylanase is confirmed by short half lives of guanidine hydrochloride inactivation.
adaptation strategy. A series of surface aromatic residues form clusters or sticky patches between pairs of molecules and these intermolecular hydrophobic interactions contribute to the enzyme's thermostability (Harris et al 1997; Connerton et al 1999). All these modifications both jointly or alone could improve the network of interactions within the protein resulting in a more stable and stiff enzyme.

Structural studies of thermal adaptation for family 10 and 11 xylanases have endorsed specific adaptation strategy identification for each family. A comparison of the thermophilic xylanases from *Thermoascus aurantiacus* and *C. thermocellum* with mesophilic family 10 xylanases specified that the thermostability in this family is a consequence of™ an improved hydrophobic packing,™ a favourable interaction of charged side chains with the helix dipoles™ increased proline content in the N-termini of helices (Leggio et al 1999).

A general thermostabilising adaptation in family 11 is a higher threonine to serine ratio as threonine has a high β forming propensity and an increased number of residues in the β strands, often an additional β strand B1 at the N-terminus (Hakulinen et al 2003). The structural differences between the families are the basis for the difference in adaptation strategies. Family 10 enzymes have a high α helix content of approximately 40% (Derewenda et al 1994) whereas family 11 enzymes have a high β sheet content of greater than 50% (Hakulinen et al 2003).

1.2.3.1.2 Psychrophiles

Only a small number of cold adapted or psychrophilic xylanase producers have been known irrespective of the most abundant cold temperature environments on earth (Sheridan et al 2000). Two gram negative bacteria *Pseudoalteromonas haloplankis* TAH3 (Collins et al 2002; Collins et al 2003; 2005)