TITLE: XYLOSE-ISOMERASE FROM *Piromyces* sp. E2: CHARACTERIZATION AND PROTEIN ENGINEERING BY CIRCULAR PERMUTATION

AUTHORS: [1]BARRETO, M.Q.; [12]WARD, R.J.

INSTITUTION: [1]Universidade de São Paulo, Programa de Pós-Graduação em Bioquímica, Faculdade de Medicina de Ribeirão Preto (Avenida Bandeirantes 3900, Prédio Central da Faculdade de Medicina, 14041-900, Ribeirão Preto, SP, Brasil); [2]Universidade de São Paulo, Ribeirão Preto, SP, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (Avenida Bandeirantes, 3900, Departamento de Química, Bloco 18, Sala 08, 14041-900, Ribeirão Preto, SP, Brasil)

ABSTRACT

Xylose-isomerase (XI) is an enzyme responsible for the conversion of D-xylose into D-xylulose, which enters the pentose-phosphate pathway. The XI from *Piromyces* sp. E2 (PyrXI) has been extensively used to engineer *Saccharomyces cerevisiae* strains in order to improve ethanol production from lignocellulose. However, the use of this enzyme in *S. cerevisiae* has been inefficient as a high number of sequence copies is necessary since *in vivo* assays proved this enzyme has a low catalytic efficiency. This work aimed at characterizing a recombinant version of PyrXI expressed in an *E. coli* system and initiate a protein engineering program of this enzyme by using circular permutation. The *xylA* gene from *Piromyces* sp. E2 was synthesized and cloned into pET28a+ for expression in *E. coli*. The polypeptide was purified by affinity chromatography, desalted and activity was tested using the sorbitol-dehydrogenase-coupled assay. Circular dichroism and dynamic-light scattering were also performed in order to analyze PyrXI folding in solution. Circular permutation was performed by circularizing the *xylA* gene in a ligation reaction for 48h. Circular fragments were concentrated with ethanol and randomly relinearized in a reaction with DNAse I, followed by blunting with T4 polymerase and subsequently cloned into pJET-blunt vector. *E. coli* DH10B cells were transformed with this library and positive clones screened. PyrXI was successfully expressed in *E. coli* and the analysis of its structure proved its monomers fold into an αβ-barrel and form a tetramer. The optimal activity was observed under pH 7.5 and 65°C in the presence of Co^{2+} and Mn^{2+} ions. A library of circular-permutated sequences was generated and clones containing an insert were selected to be used in a next round of cloning of these sequences into an expression vector. In conclusion, our results demonstrate the XI enzyme has optimal activity at 65°C in the presence of Mn^{2+}. PyrXI has a 10-fold higher activity at 65°C than 30°C, which may explain its low catalytic efficiency in *S. cerevisiae* cultured at 30°C with Mg^{2+} in solution. We are currently screening a library of circularly-permutated sequences in order to re-clone them into an expression vector and screen for enzymes with an enhanced conversion of D-xylose in *S. cerevisiae.*