Starch-binding domain for scaffold in the construction of a combinatorial library.

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Introduction. The starch binding domain of the *Lactobacillus amylovorus* amylase include five identical modules, with the capacity to bind insoluble raw starch [1]. These modules has been classified as Carbohydrates binding modules form the family 26 (CBM26) for its primary structure (www.cazy.org).
These domains have a β-sandwich structure highly stable, which can be used as a platform [2] to search for proteins with improved adsorption to its substrate. A SBD_{tag} derived from the *L. amylovorus* starch binding protein has been tried out in the development of important biotechnological applications, such as: the immobilization of recombinant proteins on starch and its use on purification systems, stabilization and use of the polysaccharide as a carrier for oral administration of antigens and therapeutic proteins [3] (Guillén, 2013).
In order to understand and improve the characteristics of these domains, we studied the potential diversification of CBM26 domain by introducing random mutations in the binding site to obtain variants with improved affinity or selectivity starch.

Methods. We performed the prediction of the tertiary structure of a CBM26 of *Lactobacillus amylovorus* with the program I-Tasser [4]. From the analysis of this model we selected 5 residues of the binding site (Y18, Y20, Q68, E74 and F77) to mutagenize and to place any of the 20 aminoacids. Mutations were introduced into seven oligonucleotides by overlapping PCR (8 cycles, 95 ° C for 1 min, 56 ° C for 2 min, 68 ° C for 2 min), the product of this amplification was re-amplified with pQ*Nco*I and pQ*Bam*HI primers (30 cycles, 98 ° C for 30 s, 59 ° C for 30 s, 72 ° C for 30 s), the library was cloned into the phagemid pG8SAET to display the library in bacteriophage M13 [5]. Variants will be selected for its capacity to bind soluble and insoluble starch.

Results. *L. amylovorus* CBM26 shows a β-sandwich three-dimensional structure (Fig. 1). In this predicted model, we could estimate the orientation of the aminoacids in the binding site to select five residues to introduce random mutations and to generate diversity in the CBM26 library. A library of 1.5 x 10^6 transformants was obtained. Twenty colonies were randomly selected and analyzed, 90% of these colonies had the desired fragment.
The sequences of the CBM26 in the selected colonies are show in Fig 2. All CBM26 tested carried at least one mutation that code for a different aminoacid in the starch binding domain. The obtained library will be display in phage M13 and analyzed for affinity and specificity to soluble and insoluble starch.

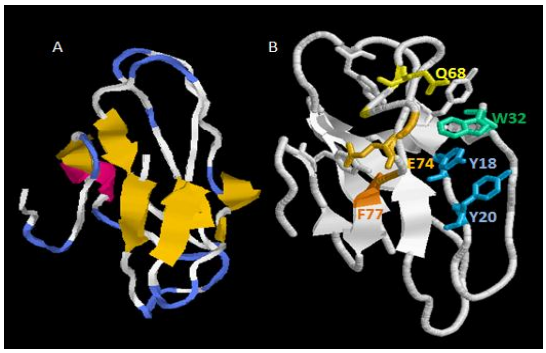

Figure 1. A) Tertiary Structure prediction of the CBM26 domain with the I-Tasser program. B) Aminoacids selected for library construction.

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C2   ICTLXTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAKVENKNTNKAITS 60
C5   ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAEVFNKNTNKAITS 60
C3'  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAYVGNKNTNKAITS 60
C8   ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAAVFNKNTNKAITS 60
C11  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAPVLNKNTNKAITS 60
C15  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAHVPNKNTNKAITS 60
C12  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAKVDNKNTNKAITS 60
C19  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAFVPNKNTNKAITS 60
C13  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAIVPNKNTNKAITS 60
C10  ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAQVLNKNTNKAITS 60
C1   ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAPVANKNTNKAITS 60
C7   ICTLGTLLISGGVTPAANAAQHDDHGTTETKKVYFEKPSSWGSRVYAKVRNKNTNKAITS 60
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C2   AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKFTPAANFAGFTFTADATYDQNGV 120
C5   AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKFTPAANFAGFTFTADATYDQNGV 120
C3'  AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKPTPAANLAGRTFTADATYDQNGV 120
C8   AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKITPAANRAGGTFTADATYDQNGV 120
C11  AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKYTPAANFAGFTFTADATYDQNGV 120
C15  AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKGTPAANFAGVTFTADATYDQNGV 120
C12  AWPGQKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKLTPAANFAGITFTADATYDQNGV 120
C19  AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKITPAANFAGFTFTADATYDQNGV 120
C13  AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTDGTKETPAANYAGSTFTADATYDQNGV 120
C10  AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFTEWDEDTSSCGRSDIHSRCHLPKWCRKI 120
C1   AWPGKKMTALGN--TSMNWISTLMKMTLILLYSPMGLRGHQQLILQVGHSQQMPLMTKMV 118
C7   AWPGKKMTALGNDKYELDLDTDEDDSDLAVIFT-DGTKSTPALIWQVVHSQQMPLMTKMV 119
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Figure 2. CBM26 sequences of 12 selected colonies, in red shows the modified aminoacids in the construction of the library.

Conclusions.
A combinatorial library was obtained from a carbohydrate binding module form the family CBM26.
The library size (1.5 x 10^6transformants) is suitable for the biopanning through interaction with soluble and insoluble starch.

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