Mapping of amino acid residues involved in the thermal stability of a beta-glucosidase

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Introduction and Aims: Sfbglu is a digestive beta-glucosidase from the fall armyworm Spodoptera frugiperda. Previous studies approached its substrate specificity and pH optimum modulation. In this present project random mutations were distributed in the primary structure of Sfbglu constituting a mutant library from which a screening based on thermal stability shall give insight on its amino acid residues responsible for this property.

Results and Conclusion: In order to validate a method for large scale screening of thermal stability, eighty single colonies of E. coli NovaBlue DE3 expressing the wild-type Sfbglu were cultivated in 96 deep wells plates. After that, bacteria were harvested by centrifugation in the same plates and disrupted by freeze-thaw cycles and lysozyme incubation. The eighty supernatants containing Sfbglu were recovered after centrifugation and each of them divided in two samples which were submitted to different thermal treatments. The first sample was incubated at 50°C for 10 min, whereas the second one was hold at 4°C. Following that these samples were tested for enzymatic activity using p-nitrophenyl beta-D-fucopiranoside in 96 wells plates. Based on these assays the ratio of activity (v₄/v₅₀) of the native Sfbglu versus the sample submitted to thermal inactivation at 50°C was calculated. This entire procedure was repeated three times, each of them with eighty colonies, showing v₄/v₅₀ of 2.3 ± 0.3, 1.9 ± 0.2 and 1.6 ± 0.2. In conclusion, the low average variance (13%) indicates that it is feasible to employ this procedure in large scale screening of thermal stability of random mutant Sfbglu. In this case, Sfbglu showing v₄/v₅₀ outside the range formed by the average v₄/v₅₀ for the wild-type Sfbglu ± 2.5 standard deviations probably exhibits an altered thermal stability.

Keywords: beta-glucosidase, thermal stability, Spodoptera frugiperda

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