Abstract

Industrial enzymes should have specific activity and thermal resistance, and new keratinases should have high keratolytic activity with a specific substrate. Due to the limited ability of available industrial keratinases, despite the ability of thermal resistance to decompose Keratin, improving specific activity with various properties by protein engineering is a quick way to obtain optimal keratinase Protein engineering, such as a mutation in a particular region or a non-specific region, is one of the techniques that is needed to obtain an enzyme. Therefore, this study was conducted to evaluate the effect of different types of mutations on the keratinase activity of Bacillus sp. Fum-120. At first, the isolate FUM 120 that separated from poultry farms in Mashhad by microbiology department of Ferdowsi University of Mashhad was evaluated quantitatively and quantitatively and identified using biochemical and molecular tests, which was determined using 16sRNA primer that this strain with 98% similarity is Bacillus. Optimum culture conditions were used for this strain, which was previously evaluated in microbiology department of Ferdowsi University. DNA was extracted from bacteria with designed primers, and then purified to reproduce the desired gene for the PCR process. PET28a+, an appropriate vector was prepared and the keratinase gene was cloned into E. coliDH5α bacteria. First, mutations were made in different and non-specific regions and cloned into E. coliDH5α , and then mutations in the Tyr114 region that were present in the active region of the enzyme were created And PET28a+ vector carrying the keratinase gene was transferred to E. coliDH5α separately. The effect of different types of mutations on the activity of bacterial keratinase and, its effect on the thermal keratinase tolerance is investigated. In this study, it was found that random mutation and mutation in the Tyr114 region, as well as a combination mutation resulting from the mixing of two mutations in a specific and non-specific region, could improve the catalytic and hydrolysis potential, thus producing a variety of high-activity keratinases. The results showed that the reduction of spatial deterrence and hydrophilia in the active region is an important effect that allows the keratinase to be more potent to decompose Keratin supplements. These findings are not only an awareness of the mutation in the particular region but also important in protein engineering and keratinase activity.

Keywords: Poisonous waste, keratinase, mutation, genetic saturation.

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Graduate school

Faculty of science

Department of Biology

The Thesis submitted for the Degree of M.Sc

(in the field of Genetic)

Title:

The effect of mutation on *Bacillus sp. Fum-120* keratinase

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Summer 2019