ISOLATION, MOLECULAR CHARACTERISATION AND IMPROVEMENT OF PECTINASE GENE(S) FROM LOCAL BACTERIAL ISOLATES

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006

M.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2016

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ABSTRACT

Nagwa Mohamed Abd El-Aziz Aly. Isolation, Molecular Characterisation and Improvement of Pectinase Gene(s) From Local Bacterial Isolates. Unpublished PhD Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2019.

In this study, twenty two bacterial isolates were isolated from agricultural crop waste soil at National Research Center. After optimization conditions using different parameters to test pectinase specific activity for high seven and two leaky isolates. The maximum pectinase specific active isolates were No. 2, 7 and 22 which recorded 71.10, 79.98 and 91.45 U/ml, respectively. Biochemical identification depended on morphological, physiological and biochemical parameters for these isolates No.2, 7 and 22 was confirmed with molecular identification by PCR amplification and sequencing of 16S rRNA gene, identified as; *Bacillus filamentosus* N2, *Bacillus endophyticus* N7 and *Klebsiella oxytoca N22.*These most three productive identified strains were treated in third step mutagenesis with ethedium bromide (Eth.Br.) and hydrogen peroxide (H_2O_2) for creating super productive mutants. After optimization conditions using different parameters to test pectinase specific activity of hyper six mutants resulted from third step mutations experiments, the maximum mutant in pectinase specific activity was *klebseilla oxttoca* H6 which recorded (185.8 U/ml). Nine pectinase genes from strain *Klebsiella oxytoca* N22 and its high mutant *Klebsiella oxytoca* H6 were isolated and completely sequenced. Strain *Klebsiella oxytoca* N22 sequences results showed that all pectinase encoding genes showed high similarity with the reference strain *Klebsiella oxytoca* jko3.Mutant *Klebsiella oxytoca* H6 sequences results showed that there were some pectinase encoding genes showed low similarity and some pectinase encoding genes showed high similarity with the reference strain.

Key words: Pectinase, pectinase specific active isolates, 16s RNA gene, third step mutagenesis, mutants, pectinase genes.

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