GENETIC CONSTRUCTION OF SOME BACTERIAL STRAINS FOR SOME PESTICIDES BIODEGRADATION

By

GHADA MOSTAFA EL-SAYED MOHAMMED

B.Sc. Science (Microbiology and Chemistry), Zagazig University, 2004

M.Sc. Agric. Sci. (Genetics), Ain Shamus University, 2013

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This thesis for Ph.D. degree has been approved by

Dr. Mohamed Serag El-Din Abd El-Sabour ………………..

Prof. Emeritus of Genetics, Faculty of Agriculture, Benha University.

Dr. Khaled Abd El-Aziz Soliman ....

Prof. of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Ashraf Bakry Abd El-Razik ……………….

Prof. of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Samir Abd El-Aziz Ibrahim ……………….

Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University.

Date of Examination: / / 2018

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Under the supervision of:

Dr. Samir Abd El-Aziz Ibrahim

Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University (Principal supervisor).

Dr. Ashraf Bakry Abd El-Razik

Prof. of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Nivien Abd El-Rahman Abosereih

Researcher Prof. of Genetics, Department of Microbial Genetics, National Research Center.

ABSTRACT

Ghada Mostafa El-Sayed Mohammed: Genetic Construction of Some Bacterial Strains for Some Pesticides Biodegradation. Unpublished PhD Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2018.

Screening the most persistent pesticides residues in five Egyptian agriculture soils reveled that organophosphorus pesticides; diazinon and chlorpyrifos were the most persistent pesticides in the Egyptian soil samples. The highest concentration (0.09 mg/kg soil) was recorded by chlorpyrifos in Kaliopia and (0.08 mg/kg soil) was recorded by diazinon in Beheira. Four potent degradative bacterial isolates were isolated from organophosphates contaminated soils and molecularly identified by PCR amplification and sequencing of 16s rDNA and groEl genes as *Cronobacter muytjensii* strain GH10, *Pseudomonas aeruginosa* strain GH2NO8, *Achromobacter xylosoxidans* strain GH9OP and *Pseudomonas putida* strain GH4SNO/P. These bacterial isolates were able to degrade 92.59%, 91.82%, 97.75% and 90.78% of diazinon (600 mg/l), for the previous mentioned bacterial strains, respectively as compared to 4.28% in control; however, they degraded 93.43%, 78.51%, 93.18% and 95.36% of chlorpyrifos (480 mg/l) as compared to 4.28% in control after 20 days of incubation. Due to *Pseudomonas aeruginosa* strain GH2NO8 and *Achromobacter xylosoxidans* strain GH9OP ethylmethane sulphonate mutagenesis, higher degrader mutants were obtained; PAM8 was able to degrade 62.19% of diazinon and 54.08% of chlorpyrifos as compared with *Pseudomonas aeruginosa* as wild type that degraded 38.19% and 29.44 % of diazinon and chlorpyrifos, respectively. However AchM15 exhibited the ability to degrade 84% of diazinon and 63% of chlorpyrifos as compared to 49% and 34% in *Achromobacter xylosoxidans* as wild type respectively after five days of incubation. PAM8 and AchM15 were subjected to second step EMS mutagenesis, PAMS9 and AchMs1 were the best mutants; they degraded 86.21% and 96.03% of diazinon and

75.70% and 70.28% of chlorpyrifos after five days of incubation. Genes of *oph* in *Cronobacter muytjensii* strain GH10 and *Achromobacter xylosoxidans* strain GH9OP were cloned into pET29a (+) vector and transformed into *E. coli* DH5α. DNA sequences of *oph* genes in these bacterial isolates were deposited in Genbank under accession number MH018245 and MH018244, respectively. Moreover these genes were successfully expressed in *E.coli* BL21 (DE3) as expression host. IMPH and TCP as diazinon and chlorpyrifos metabolites were detected due to bacterial biodegradation by GC/MS analysis.

Key Words: Organophosphates, Microbial biodegradation, 16sr DNA, groEl; EMS mutation; *oph* genes; *E. coli*; gene cloning and expression; GC/MS analysis.

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