GENETIC IMPROVEMENT OF SOME BACTERIAL STRAINS PRODUCING VITAMIN B_{12}

By

Bigad El-Sayed Mahmoud Ahmed Khalil
B.Sc. Agric. Sci. (Biochemistry), Ain Shams University, 2002
M.Sc.Agric.Sci.(Genetics), Ain Shamus University, 2011

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

Dr. Mohamed Abdel- Baith El- Seehy ..……..…...
Prof. Emeritus of Genetics, Faculty of Agriculture, Alexandria University

Dr. Khaled Abdel-Aziz Abdel-Aty Soliman ……..
Prof. of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Ashraf Bakry Abdel-Razik ……..
Prof. of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Samir Abdel-Aziz Ibrahim ……..
Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University

Date of Examination:

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Under the supervision of:

Dr. Samir Abdel-Aziz Ibrahim
Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University (Principal supervisor)

Dr. Ashraf Bakry Abdel-Razik
Prof. of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Abd El-Hamid Abd El-Alim Haggran
Researcher Prof. of Genetics, Department of Microbial Genetics, National Research Center

ABSTRACT

Bigad El-Sayed Mahmoud Ahmed Khalil: Genetic Improvement of Some Bacterial Strains Producing Vitamin B_{12}. Unpublished PhD. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017

The present investigation aims to improving some bacterial strains for producing vitamin B_{12} through genetic improvement methods *i.e* induction of mutation and protoplast fusion. In this study 18 bacterial cultures were isolated from three different areas (Menofia - Domiatt-Cairo) and tested for production of vitamin B_{12}. Three isolates which highly producing vitamin B_{12} are identified as a Pseudomonas strains by some biochemical tests and 16s rDNA technique. The B_{12} production was determined by spectrophotometric and HPLC methods. Multiple mutagenesis technique by Ethyl Methan Sulphonate (EMS) as a chemical mutagen and Ultraviolet (UV) as a physical mutagen used to increase vitamin B_{12} production of strains. The strains treated with 200 mM EMS for different exposure time (15, 30, 45, 60 and 90 min). Mutant M 6 from (*P. aeruginosa*) produced 6 mg/L B12 higher than their parent that produced 4 mg/L that increase the production by 50% in the first step mutation, in the second step mutation the mutant M 6.9 from (*P. aeruginosa*) produced 12.3 mg/L that increase the production by 207%, and in the third step mutation the mutant M 6.9.9 from (*P. aeruginosa*) produced 15.5 mg/L that increase the production by 287%.

The strains were treated with UV with different exposure time (30, 60, 90, 120, 150 and 180 sec) at fixed distance of 15 cm from light source, mutant coded P1 (M6.9.9.uv7), giving 20.3 mg/L that increase the production by 408 % in first step mutation. In in the second step mutation, the mutant coded P1 (M6.9.9.uv7.5) was the most B_{12} producer mutant,

giving 21 mg/L showed 425% improvement over the wild-type strain (P1). Intra and inter specific protoplast fusion between auxotrophic mutants isolated after treatment with mutagen was carried out, twenty fusants were successfully obtained, fusant F10 produced 7 mg/L, from intra specific protoplast fusion, fusant F4 produced 7.9 mg/L, from inter specific protoplast. Finally ISSR-PCR marker technique were used to differentiation among parents and its mutants.

Keywords: *Pseudomonas*, vitamin B_{12}, mutation induction, EMS, UV, ISSR.

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