

**GENE TRANSFER BETWEEN SOME
LYSOGENIC STRAINS OF *Bacillus* sp.**

BY

Σ-10/29.. / 2

NAHLA REFAAT MOHAMED REFAI

**B. Sc. Agric Sci. (Genetics)
1989**

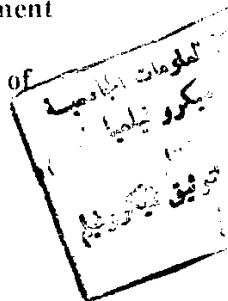
Faculty of Agric., Ain Shams University, 1989

536.0821
N. R.

64125

Thesis submitted in partial fulfillment
of
the requirements for the degree of

**MASTER OF SCIENCE
in
AGRICULTURE
(GENETICS)**



**Department of Genetics
Faculty of Agriculture
Ain Shams University**

1996

APPROVAL SHEET

**GENE TRANSFER BETWEEN SOME
LYSGENIC STRAINS OF *Bacillus* sp.**

By

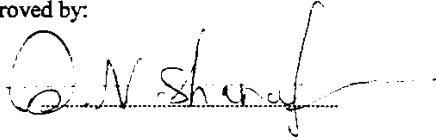
NAHLA REFAAT MOHAMED REFAI

B. SC. Agric. Sci. (Genetics), 1989

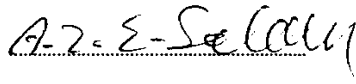
Faculty of Agric., Ain Shams University, 1989

This thesis for M.Sc. degree has been approved by:

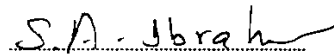
Prof Dr. A.N. El-Sayed Sharaf
Prof. of Genetics, Fac. of Agric.,
Cairo University



Prof. Dr. A.Z. El-Abidin Salam
Prof. of Genetics, Fac. of Agric.,
Ain Shams University



Prof. Dr. S.A. Ibrahim
Prof. of Genetics, Fac. of Agric.,
Ain Shams University



Date of examination: 22/7/1996



GENE TRANSFER BETWEEN SOME LYSOGENIC STRAINS OF *Bacillus* sp.

BY

NAHLA REFAAT MOHAMED REFAI

B. Sc. Agric Sci. (Genetics), 1989

Faculty of Agric., Ain Shams University 1989

Under the Supervision of

Prof. Dr. Samir Abd El-Aziz Ibrahim

Prof. of Genetics, Fac. of Agric.,
Ain Shams University.

Dr. Mamdouh Kamal Amin

Assistant Prof. of Genetics, Fac. of Agric.,
Zagazig University.

Dr. Fatma Mohamed Ibrahim Badawy

Lecture of Genetics, Fac. of Agric.,
Ain Shams University.

ABSTRACT

Nahla Refaat Mohamed Refai, Gene Transfer Between Some Lysogenic Strains of *Bacillus* sp., Unpublished Master of Science, Genetics, Fac. of Agric, Ain Shams Univ., 1996

The main aim of the present investigation is evaluate the efficiency of transduction in transfer of different genetic markers between different lysogenic species as well as different strains of *Bacillus* including *B. thuringiensis*. In order to achieve this goal, induction of mutation, identification and evaluation of such mutant were carried out. Transduction experiment were done between *B. thuringiensis* WT and its mutants, *B. subtilis* WT and its mutants and between *B. thuringiensis* mutants and between *B. subtilis* mutants. To evaluate transductant electrophoretic protein banding patterns and crystal formation were studied for some of isolated transductants, mutants and the wild type. The results indicate that the transduction is an efficient method for gene transfer in bacteria under investigation.

Key words: Gene transfer, *Bacillus*, Transduction, UV irradiation, Electrophoresis..

ACKNOWLEDGMENT

By the name of Allah the most merciful and compassionate.

I gratefully acknowledge my deep indebtedness to Dr. S.A. Ibrahim professor of Genetics, Faculty of Agriculture, Ain Shams University for supervising this work.

I wish to express particular thanks to Dr. Fatma M.E. Badway Lecture of Genetics, Faculty of Agriculture, Ain Shams University for very kindly helping me with regard to my thesis. My very sincere thanks go to Dr. M.K. Amin Assistant Professor of Genetics, Faculty of Agriculture, El-Zagazig University for his help.

Deep thanks with special great to Dr. R.A. El-Adway, Professor of Genetics, Om-Al-Quoraa University, Saudi Arabia for helping and supporting me.

Thanks for all the members of the Genetics Department, Faculty of Agriculture, Ain Shams University.

I offer my sincere gratitude for my parents and sisters reinforcing and praying to me supporting with deep love. Deep thanks with great for my husband for all of his helping. Finally, my first thanks go to Allah for supporting me in life.

CONTENTS

	Page
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	3
1. INDUCTION OF MUTATIONS	3
2. TRANSDUCTION.....	5
3. BIOCHEMICAL GENETIC ASPECTS IN DETECTION AND IDENTIFICATION OF MICROORGANISMS.....	7
MATERIALS AND METHODS.....	11
1. MATERIALS.....	11
1.1. Bacterial strains.....	11
1.2. Bacteriophage.....	11
1.3. Media.....	11
1.3.1. Yeast extract peptone medium (YEPD):.....	11
1.3.2. Minimal medium (MM):.....	11
1.3.3. Sporulation medium (SM):.....	12
1.3.4. Media for protopasting:.....	12
1.3.5. Specific medium (SpM):.....	12
1.4. Buffers and stains:.....	12
1.4.1. Phosphate buffer (PhB):.....	12
1.4.2. Phage buffer (PB):.....	13
1.4.3. DTT/urea buffer:.....	13
1.4.4. Electrophoretic buffer:.....	13
2. METHODS.....	14
2.1. Induction of mutations.....	14
2.1.1. Mutagenic treatment:.....	14
2.1.2. Isolation and identification of mutants:.....	14
2.2. Transduction:.....	15
2.2.1. Phage preparation:.....	15
2.2.2. Spot test:.....	15
2.2.3. Transduction procedure:.....	15
2.3. Protein analysis.....	16
2.3.1. Protein extraction:.....	16
a. Preparation of protoplasts.....	16
b. Solubilization of coat proteins:.....	16
2.3.2. Gel preparation:.....	16
a. Resolving gel (RG):.....	17
b. Stacking gel (SG):.....	17
2.3.3. Polyacrylamide gel SDS electrophoresis:.....	17
2.4. Microscopic examination of crystals:.....	18
2.4.1. Crystal preparation:.....	18
2.4.2. Staining procedure of <i>B. thuringiensis</i> crystalals:.....	18
Examination for the speed of sporulation:.....	19
RESULTS AND DISCUSSION.....	20
1. MUTATION STUDIES.....	20
1.1. Induction of mutations in both <i>B. thuringiensis</i> and <i>B. subtilis</i>	20

1.2. Isolation and identification of mutant colonies.....	22
2. PHAGE MEDIATED GENE TRANSFER (TRANSDUCTION).....	23
2.1. Intraspecific transduction of single genes:.....	24
2.2. Interspecific transduction of single genes:.....	25
2.3. Transduction recombination and linkage map of some lysogenic strains of <i>B. thuringiensis</i> and <i>B. subtilis</i> by phage $\phi 63$	27
2.4. Co-transduction genetic map.....	28
2.4.1. Mapping of <i>glu met- trp</i> genes in <i>B. thuringiensis</i> by phage $\phi 63$:.....	28
b- Mapping of <i>met trp lys</i> genes in <i>B. subtilis</i> by phage $\phi 63$:.....	29
3. PROTEIN BANDING PATTERNS.....	30
3.1. Effect of UV mutagenesis on the vegetative cells protein banding patterns of <i>B. thuringiensis</i>	30
3.2. Effect of UV mutagenesis on the protein banding patterns of sporulated <i>B. thuringiensis</i>	34
3.3. Effect of transduction via phage $\phi 63$ on <i>Bacillus</i> species protein banding pattern:.....	36
3.3.1. Effect of transduction on vegetative cells protein banding pattern:.....	36
3.3.2. Effect of transduction on spore cells protein banding patterns.....	44
4. GENETIC MODIFICATION IN RELATION TO PARASPORAL CRYSTAL PRODUCTION:..	53
4.1. The effect of UV irradiation on the formation of parasporal crystal in <i>B. thuringiensis</i> :.....	53
4.1.1. Relative concentration of crystals.....	54
4.1.2. Relative size of crystals.....	54
4.2. The efficiency of transduction in transferring the gene(s) controlling crystal formation from <i>B. thuringiensis</i> to <i>B. subtilis</i>	56
4.3. Examination of toxicity to Lepidoptera larvae:.....	59
SUMMARY.....	61
REFERENCES.....	66
ARABIC SUMMARY.....	

LIST OF TABLES

	Page
1. Bacterial strains which used and their sources	11
2. Survival percentage of both <i>B. thuringiensis</i> and <i>B. subtilis</i> after applying different exposure time to UV light.	21
3. Isolated mutants and their nutritional requirement.	23
4. Intraspecific gene transfer of some nutritional marker genes via transduction by phage $\phi 63$ between <i>B. thuringiensis</i> strains using different single mutants as a recipient and the wild type as a donor.	24
5. Intraspecific gene transfer of some nutritional marker genes via transduction by phage $\phi 63$ between <i>B. subtilis</i> strains using Bs 3 mutant strain (<i>lys⁻ met⁻ trp⁻</i>) as a recipient and the wild type as a donor.	25
6. Interspecific gene transfer of some nutritional marker genes via transduction by phage $\phi 63$ between <i>B. thuringiensis</i> wild type as a donor and <i>B. subtilis</i> strain Bs 3 (<i>lys⁻ met⁻ trp⁻</i>) as a recipient.	26
7. Interspecific gene transfer of some nutritional marker genes via transduction by phage $\phi 63$ between <i>B. subtilis</i> wild type as a donor and <i>B. thuringiensis</i> strain Btuv3 (<i>glu⁻ trp⁻</i>) as a recipient.	26
8. Cotransduction of some nutritional marker genes via transduction by phage $\phi 63$ using <i>B. thuringiensis</i> wt as a donor and its mutant (<i>trp⁻ glu⁻</i>) as a recipient and using <i>B. subtilis</i> wt as a donor and its mutant (<i>trp⁻ met⁻ lys⁻</i>) as a recipient.	27
9. Cotransduction and genetic map of the genes <i>trp</i> , <i>met</i> , <i>glu</i> in <i>B. thuringiensis</i> obtained from cross Btuv4 (<i>glu⁺ met⁺ trp⁺</i>) as a donor x Btuv3 (<i>glu⁻ met⁻ trp⁻</i>) as a recipient via phage $\phi 63$	29
10. Cotransduction and genetic map of the genes <i>met</i> , <i>trp</i> , <i>lys</i> in <i>B. subtilis</i> obtained from cross <i>B. subtilis</i> wild type as a donor x Bs 3 (<i>met⁻ lys⁻ trp⁻</i>) as a recipient via phage $\phi 63$	30
11. Comparison of protein banding patterns of vegetative cell water soluble protein between <i>B. thuringiensis</i> and some auxotrophic mutants based on the numbers and locations of bands.	31
12. Comparison of protein banding patterns of spore cells water soluble protein between <i>B. thuringiensis</i> and some auxotrophic mutants based on the numbers and locations of bands.	33
13. Comparison of vegetative cells protein banding patterns of four transductants and their parents, the donor Bs wt and the recipient BtUV3.	36
14. Comparison of vegetative cells protein banding patterns of two transductants and their parents, the donor Bs wt and the recipient BtUV2.	38
15. Comparison of vegetative cells protein banding patterns of two transductants and their parents, the donor Bs wt and the recipient BtUV4.	40
16. Comparison of vegetative cells protein banding patterns of two transductants and their parents, the donor Bs wt and the recipient BtUV1.	42

17. Comparison of spore cells protein banding patterns of four transductants and their parents, the donor Bs wt and the recipient BtUV3.....	44
18. Comparison of spore cells protein banding patterns of two transductants and their parents, the donor Bs wt and the recipient BtUV2.....	46
19. Comparison of spore cells protein banding patterns of two transductants and their parents, the donor Bs wt and the recipient BtUV4.....	49
20. Comparison of spore cells protein banding patterns of two transductants and their parents, the donor Bs wt and the recipient BtUV1.....	51
21. Relative concentrations of parasporal crystal of the <i>B. thuringiensis</i> wt and some of mutant isolates.....	52
22. The speed of sporulation of <i>B. thuringiensis</i> and <i>B. subtilis</i> and some transductants.....	54
23. The efficiency of <i>B. thuringiensis</i> , <i>B. subtilis</i> , the big crystal mutant Btuv3 and the two of transductants T1 (Bs 3), T2 (Bs 3) as biological pesticide on Lepidoptera larvae, measured as percentage and speed of mortality.....	56

LIST OF FIGURES

	Page
1. Survival percentage of both <i>B. thuringiensis</i> and <i>B. subtilis</i> after applying different exposure time to UV light.....	21
2. Zymogram of protein banding patterns of <i>B. thuringiensis</i> wild type and its mutants in the vegetative stage.....	32
3. Zymogram of protein banding patterns of <i>B. thuringiensis</i> wild type and its mutants in the sporulation stage.....	34
4. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and Btuv3 (<i>glu⁻ trp⁻</i>) as a recipient in the vegetative stage.....	37
5. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and Btuv1 (<i>glu⁻</i>) as a recipient in the vegetative stage.....	39
6. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and BtUV2 (<i>trp⁻</i>) as a recipient in the vegetative stage.....	41
7. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and BtUV4 (<i>met⁻</i>) as a recipient in the vegetative stage.....	43
8. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and BtUV3 (<i>glu⁻ trp⁻</i>) as a recipient in the sporulation stage.....	45
9. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and BtUV4 (<i>met⁻</i>) as a recipient in the sporulation stage.....	47
10. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and BtUV1 (<i>glu⁻</i>) as a recipient in the sporulation stage.....	48
11. Zymogram of protein banding patterns of some transductants resulted from the cross <i>B. subtilis</i> wild type as a donor and BtUV2 (<i>trp⁻</i>) as a recipient in the sporulation stage.....	50
12. Effect of UV on the number and size of crystals.....	53
13. Effect of transduction on the number and size of crystals.....	55

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

INTRODUCTION

The group of biosystems especially which are specified as pathogenic microorganisms for insects is varied and diverse. Among these, there are a broad range of viruses, bacteria and fungi. Each of these subgroups is composed of a spectrum of organisms that vary in their mode and site of infection and mechanism of pathogenicity. While some pathogens exhibit rather wide host ranges, many have their preferred targets among certain insect species as well as selective pathogenicity for larval or adult life stages. This spectrum probably reflects the overlap in natural habitats of particular microorganisms and insects, the mechanism(s) of pathogenicity (nature of toxic substances produced), and the needs of the pathogen are intensively reviewed by **Aronson et al. (1986)**.

The major species of bacteria with their mechanism(s) to infect and kill uncompromised, healthy insects are spore-formation bacilli. For these species, the hemolymph of insect larvae is an excellent nutritional environment for bacterial proliferation, and sometimes for sporulation (**Burges, 1982** and **Dulmage and Aizawa, 1982**).

Among the spore-forming bacilli, there are major groups (*Bacillus thuringiensis* and *Bacillus sphaericus*) which produce protoxins during sporulation. *Bacillus thuringiensis* is a rather well studied sporeforming organism. Much of this work has been focused on the biochemical properties of the endotoxin in the crystal protein. Crystals of *B. thuringiensis* contain proteins of 125 to 140 KDa or 70 to 80 KDa (**Höfte and Whiteley, 1989**), and depending on the variety of this species. **Bauman et al. (1991)** reported that the proteins may be toxic to insects of the orders Lepidoptera (caterpillars), Coleoptera (beetles) or Diptera (mosquitoes and black flies). Upon ingestion by a susceptible species, the proteins in these crystals are solubilized in the midgut by a combination of alkaline pH and proteolysis and become toxic (**Tajo and Aizawa, 1983** and **Aronson et al., 1986**). The high-molecular-weight proteins are protoxins, proteolysis in the gut leads to their conversion to lower-molecular-weight toxins (**Whiteley and Schnepf, 1986**). The sporeforming bacilli are viewed as having the highest potential for use in the management of insect pest population, and these organisms have

received the greatest commercial attention. These are currently 410 registered formulations of *B. lentimorbus* approved for use in the United States against insect pests (according to the National Pesticide International Retrieval System, Purdue University, West Lafayette, Ind.). Many of approved formulations including both fungicides and chemical insecticides have contain *B. thuringiensis* to increase their activity so that they registered for use against a spectrum of target pests for exceeding the larvae of Lepidopteran and Dipteran species susceptible to the spores and crystals of the bacterium (Aronson *et al.*, 1986).

It is worthy to note that the genetic characterization of this important organism and the requirement of a dependable DNA exchange system is essential (Barsomian *et al.*, 1984) About 20 subspecies of *B. thuringiensis* are recognized (Bullal *et al.*, 1975 and Tyrell *et al.*, 1979) and some of them are varied in their pathogenicity to various insects. Systems for genetic exchange in *B. thuringiensis* could also be of potential use for the construction of tailor-mode insect pathogens (Landen *et al.*, 1981).

For these important reasons, efforts have made to develop an efficient system for genetic exchange in such organisms. While until 1986 there are no reports of transformation of *B. thuringiensis* cells (Aronson *et al.*, 1986). Transformation of protoplasts with polyethylene glycol induced uptake of plasmid DNA has been reported (Martin *et al.*, 1981) but the frequencies were relatively low compared to those obtained with *B. subtilis* (Chang and Cohen, 1979).

There have been reports of several transducing phages in *B. thuringiensis* (Perlak *et al.*, 1979; Lecadet *et al.*, 1980 and Landen *et al.*, 1981). The lytic nature of these phages represented a great disadvantage.

The present study was planned firstly to induce mutations in order to isolate different genetic markers including those which are varied in protoxin crystals and evaluate such mutants. Finally used those identified mutants to test and evaluate the efficiency of the general transduction in transfer different genetic markers. To overcome the disadvantage resulted from lytic effect of phage, hysogenic bacteria were used.

REVIEW OF LITERATURE

1. Induction of mutations

Some of the most significant advances in microbial genetics is based on the study and use of mutations. Mutations in microorganisms can be induced either by chemical or physical mutagens.

Ultraviolet (UV), as a physical mutagen, has been employed in a great variety of microorganisms for the routine production of mutations. UV is a non ionizing form of radiation. It has several effects on DNA, but the major damage of DNA which cause the induction of mutation by UV light is the production of thymine dimers. UV was first undertaken in *E. coli* by **Demerec and Later Jet (1946)**. They showed that with UV-irradiation the rate of mutations had increased by the increasing of UV dosage till a plateau is reached followed by a slow drop in lethality of cells.

Perkins (1949) established stable biochemical mutants in the fungus *Ustilago maydis* by mean of UV irradiation.

Ultraviolet light was found to be effective in inducing both forward and reverse mutations and has been used to obtain auxotrophs in *Saccharomyces cerevisiae* (**Hawthorne and Mortimer, 1960** and **Mortimer and Hawthorne, 1966**).

Streptomyces indicus was also subjected to the induction of mutations with UV-irradiation doses (**Chakralarty, 1969**). Morphological and auxotrophic mutants were isolated. In addition, increase or complete absence of antibiotic activity in biochemical mutants were reported.

O'Sullivan and Pirt (1973) used UV to induce lysine auxotrophs in *Penicillium chrysogenum*. The process of photoreactivation was observed in a wide range of organisms, although not all, and lead to diverse effects of UV on them.

Rupert et al. (1958) demonstrated that cell free extract from *E. coli* which is a photoreactivable species, reactivated the DNA of *Haemophilus influenzae*, which shows no photoreactivation, in the presence of visible light.