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

BY

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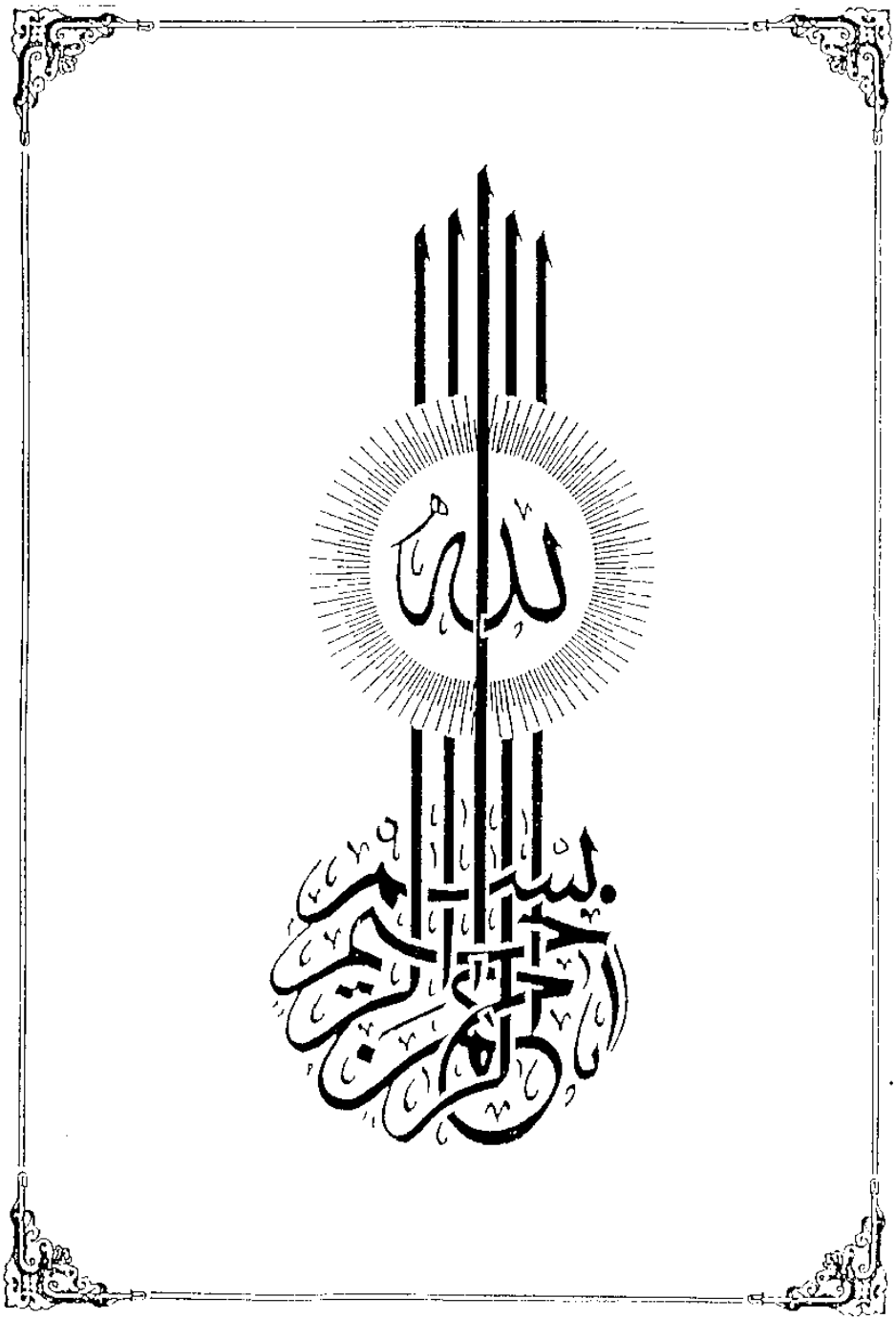

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ABSTRACT

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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..

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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. thurigiensis* 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, effects 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 cerivisiae* (**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'Sulivan 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.