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"A survey study of Bacillus thuringiensis (BT) using phages P1,P2 &P3 in Tamluk, West Bengal,India"

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Abstract

Genetically modified food[1], GM foods and crops like Bt cotton[2], Bt Brinjal[3],developed from Bacillus thuringiensis, an insecticide[4] and "Round up Ready"[5], a soybean product from other microorganism led the scientist to conclude that the future world's hunger and malnutrition can be overcomed[6]. To make more contribution and future use, a survey study of distribution of Bacillus thuringiensis is done Bt- phages[7] P1,P2,P3, are used in replica formation pattern in M-1[8] agar medium. Spores are used in this study . Only 6 phage affected colonies are detected and their pathogenocity is confirmed on Bombyx mori larva.

Key words : Bacillus thuringiensis, insecticide, pathogenicity.

Materials and methods

Sample of soil, mud and dumping water were collected from 105 different regions of West Bengal, in different sterile containers. 10 gm or 10ml of each of them was chosen and thoroughly mixed taking glass beads inside the tube and shaken well in a shaker and then the final volume was made 10ml by adding sterile water and again mixed well. Then the content was centrifuged at 2000r.p.m.for 10 minutes. Then 10ml supernatant from each of the tube was transferred in sterile tube and placed in boiling water bath for 10minutes and they were allowed to cool placing the bath in cold water container. These samples were made now ready to use as spores suspension.

Preparation of spore plates

Many decimal dilutions are made from each of the samples.

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Several marked and sterile Petri-plates were taken and in each 0.5ml of dilute and stock solution were distributed .To each of the spore inoculated plates then 20ml of 1.5gm% molten M-1 agar was added and mixed and allowed to cool. During preparation of molten M-1 agar medium 0.15ml alcohol saturated nystatin were mixed per 500ml of medium. Some uninoculated plates were also made. Same plates containing 0.5ml sterile water were also made. Those plates then served as control plates. All the plates were then placed in incubator for incubation at 37^0c.

Preparation of phage and phage plates of Bacillus thuringiensis, ATCC13366.

A water suspension of Bacillus thuringiensis was made and 0.5ml of it was added to 100ml conical flask each containing 50ml broth M-1. Such 4 conical flask and 1 without inoculum were placed in shake culture. The turbidity of them were recorded by klett at time intervals of 15 minutes. When the turbidity raised up

to 50, except the uninoculated blank (served as control), all 4 conical flasks were taken out and 3 of them were then mixed with P1,P2,P3 phages and again allowed them for shake culture. When the turbidity falls up to 25 in phage inoculated bacterial growth, the flasks were taken out. The contents of them were filtered in sterile Millipore membrane filter. The filtrates thus formed were the phage filtrates of BT phage-1, Phage-2 and phage-3. Those phages were then ready for preparation of phage-agar-M-1 plates. Those plates were made by pouring and tilting 0.5ml of each filtrate in M-1 molten agar medium at 45^0c.

Those plates were now used for transferring bacterial colonies developed in the spore inoculated plates. Growth in the spore inoculated plates was used as first mother plates for transcriptional study, the uninocullated agar plates and phage marked plates were taken and bacterial colonies from mother plates were transferred with tooth prick as follows.

Mother plates to Uninoculated plates to phage plates and then tooth prick was discarded. For each phage plate separate tooth prick was used.

All the plates were then placed for incubation at 37^0c. After 36 hrs, the plates were observed.

The colonies formed in first mother plate if grew in second plates, they were replica of the first mother plates colonies and if there was no growth of the colonies in phage plates, it was phage affective colony. The colonies which were phage affective are thought Bacillus thuringiensis colonies. Such colonies were picked up and used for pathogenocity study on Bombyx mori.

Pathogenocity study

Phage affective colonies were picked up and grown in M-1 medium at 37^0c. After that their suspension in 0.9% NaCl was made. 52 Bombyx mori, larva and mulberry leaves were collected. From sericulture research station, Baharampur, Murshidabad west Bengal India, were taken and 4 groups were made and all the leaves were washed with sterile solutions. One group of larva was allowed to take saline washed leaves only and considered as control group and the other three groups were treated with phage affective bacterial suspension. All this of them were kept at 30^0c. Then mortality was noted both in control group and treated groups. The affected larva were collected. And again they were examined for microscopic study and gram study. Total result of phage affected bacteria is shown in tabular form.

Table-1: Distribution of phage affected sparing Bacteria.

Sample collected from	No. of sample	No. of Spore/gm.	No. of colonies choosen	Phage affected colonies		
				P1	P2	P3
Soil	35	2.3×10^3	30	2	3	1
Mud	35	1.5×10^2	15	–	–	–
DumpingGround	35	2.8×10^2	10	–	–	–

• **(-)** means no phage affected colony

Table-2: Mortality of Bombyx mori larva in different phages at different times.

Treated Gr.	0 hr.	3 hrs.	6 hrs.	9 hrs.	Total
P1	–	4	3	2	9
P2	–	3	2	5	10
P3	–	4	3	2	9
Control Gr.	–	–	–	–	–

• **(–)** means no mortality occurred.

Results

Table- 1: shows among all sparing bacteria only 6 Phages affected colonies are present.

Table- 2 : Shows phage affected colonies caused the death of Bombyx Mori larva within 9hrs. They are the pathogenic Bacteria. Microscopic observation and gram staining shows that they are gram positive bacilli.

Discussion

For the determination of phage affected sporing bacteria 105 samples were collected from the locality of Tamluk, West Bengal and they were examined. The results (Table 1) shows that highest number of spores are present in the soil and a variable number are present in the mud and dumping ground. Phage affected sporing bacteria are also present in the soil sample and they were few only and the isolated numbers were 6. The isolated phages showed the same morphology and relative constant plaque sizes in sporing bacterial lawn of bacillus thuringiensis. Results also indicated among the large number of sporing bacteria, only a few were phage affected i.e all sporing soil bacteria are not affected by P1,P2,P3 i.e they are not bacillus thuringiensis. Phages P1,P2,P3 treated bacteria were able to kill the Bombyx mori larva(Table2). Majority of larva died within 9 hrs but there was no effect on the control group of Bombyx mori. Cross infectivity study with the phages P1,P2,P3 on bacillus subtilis, bacillus cereus, and bacillus pumilus and 30 isolated sporing bacillus was done. Other sporing bacteria like ATCC 13366 and it's mutant varieties.[9] (developed in the laboratory,NSM resistant to 3000micro gram/ ml. streptomycin),Bnc (resistant to 20U/ml bacitrecin), and TcSm (resistant to 20 micro gram/ml of Tetrecycline and 100 mg/ml of Streptomycin) were also used. There was plaque formation in the bacterial lawn of ATCC13366 and its mutants. Gram staining and microscopic observation including spore formation, and cross infectivity study with phages P1,P2,P3 on bacteria, and plaque formation studies on bacterial colonies from sporing bacteria, and their pathogenicity study (Table 2) suggest to conclude that bacillus thuringiensis are present in the soil and dumping ground of the locality and they might have caused the death of insects in the nature.

Conclusion

The gram positive, sporing and Bombyx mori killing bacteria are present in different localities.

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